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EFFECT OF NUTRIENTS, MEDIA, AND GROWTH SUBSTANCES ON THE GROWTH OF THE CABOT VARIETY OF VACCINIUM CORYMBOSUM [1]

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INTRODUCTION

In addition to its commercial importance (*4*) [2] the blueberry is an important source of supplemental farm income, and the upland species have excellent erosion-control value. Both wild and cultivated blueberry plants often exhibit certain symptoms which, because of the absence of a pathogen, appear to be caused, in part at least, by some nutrient deficiency. If such symptoms could be definitely ascribed to the lack of single mineral nutrients, the diagnosis and cure of these deficiencies in the field would be greatly facilitated.

It has been generally observed that a peat medium is very favorable for blueberry growth. Should some mineral deficiencies fail to appear, or appear to a lesser degree in peat medium, it might be reasonable to assume that the benefit derived from peat is due in part or in whole to the presence of these elements in available form. In such a complex of unknown deficiencies the role of growth substances must also be considered.

To attempt to solve the problem as defined, it was decided to study the behavior of the blueberry under controlled conditions of pot culture wherein the effects of nutrient solutions deficient in single elements, such as nitrogen, potassium, phosphorus, iron, sulfur, manganese, magnesium, boron, and calcium, could be compared with full-nutrient cultures. Such a procedure also permitted the superimposing of peat on the medium, as well as certain growth substances, for comparison with quartz sand. In the selection of material for study, it was decided to use clonal stock of a cultivated variety to avoid genetic variation among the plants, although it was realized that the results would not necessarily apply to all blueberry species.

Previous work of other investigators was of little aid in designing these experiments. Doehlert and Shive (*5*), using 20 nutrient combinations calculated to contain a salt concentration equivalent to one-half atmosphere, found that solutions containing high levels of nitrogen were most favorable for blueberry growth. Alexander [3]

[1] Received for publication February 21, 1941. A cooperative study by the Hillculture Division, Soil Conservation Service, U. S. Department of Agriculture, and the Department of Horticulture, University of Maryland. Scientific contribution No. 525, Maryland Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 327.

[3] ALEXANDER, T. A. Unpublished data, University of Maryland in cooperation with the Soil Conservation Service.

obtained the greatest dry weight when he used the No. 7 solution of Doehlert and Shive to which he added boric acid, manganese chloride, aluminum sulfate, and iron citrate.

The results of fertilizer experiments in the field supplied additional information on the nutrient requirements of blueberries. Good responses to liberal applications of nitrate nitrogen were obtained in New Jersey (*2*, *3*) but not in Michigan, where the soil contains appreciable amounts of organic matter (*11*). Applications of superphosphate and complete fertilizer increased the yield of blueberries both in New Jersey and in Michigan.

MATERIALS AND METHODS

On March 1, 1940, sixty 2-gallon coffee urn liners (crocks) were filled with quartz sand, and 60 with a 3-inch depth of German peat above the sand. Good drainage was obtained by covering the drain hole with glass wool. The media were then leached for 2 weeks with heavy applications of tap water.

Rooted 4-inch softwood cuttings of the Cabot blueberry (*Vaccinium corymbosum* L.) were weighed individually and placed in the media three to each liner on March 15. Tap water was added for 10 additional days until the cuttings began to leaf out, after which various nutrient solutions were applied by Dubuy's method (*6*). Twelve liners, six with peat-on-sand, and six with sand only, were used for each nutrient solution.

The full-nutrient solution contained the following chemically pure salts, based on the results of Doehlert and Shive (*5*) and Alexander: [4] 150 parts per million of monopotassium phosphate; 600 p. p. m. of calcium nitrate; 150 of magnesium sulfate; 275 of ammonium sulfate; 2.8 of boric acid; 1.8 of manganese chloride; 5 of iron citrate; and 2 p. p. m. each, at biweekly intervals, of copper and zinc sulfate.

The modifications of the full-nutrient solution, shown in table 1, were made in order to obtain the various mineral-deficient solutions.

TABLE 1.—*Modifications of the full-nutrient solution used in order to obtain the mineral-deficient solutions required for growing blueberry plants*

Mineral deficiency desired	Chemicals omitted	Chemicals substituted
Nitrogen	Calcium nitrate	Calcium sulfate.
	Ammonium sulfate	Sodium sulfate.
Potassium	Monopotassium phosphate	Monosodium phosphate.
Calcium	Calcium nitrate	Potassium nitrate.
Magnesium	Magnesium sulfate	Sodium sulfate.
Sulfur	Magnesium sulfate	Magnesium chloride.
	Ammonium sulfate	
Phosphorus	Monopotassium phosphate	Potassium chloride.
Boron	Boric acid	
Iron	Iron citrate	
Manganese	Manganese chloride	

Once weekly, 2 of the sand cultures and 2 of the peat-on-sand cultures of each of the 10 treatments received 500 ml. of 1×10^{-8} thiamin chloride (vitamin B_1) solution; similarly, 2 sand cultures and 2 peat-on-sand cultures of each treatment received 500 ml. of 1×10^{-6} thiourea; distilled water was used as a check. These applications,

[4] See footnote 3, p. 313.

besides supplying the substances, also served to leach out any salt that might have accumulated during the week. Since the tap water contained considerable quantities of nutrients,[5] distilled water was used after April 1.

Between April 29 and 30 one of the three plants in each liner was removed. The roots were washed free of adhering sand and peat particles, allowed to drain on a wire screen for several minutes, and weighed. After the total fresh weight had been obtained the current growth was pruned off and weighed separately. All the plant parts were then placed in individual manila bags and dried in an oven at 70° C. for 3 to 4 days until constant in weight. A second plant from each liner was removed between June 10 and June 14 and treated similarly. The full-nutrient solution was then applied to all the remaining blueberry plants (one plant in each container) with the exception of those not receiving manganese. Nutrient-deficient solutions were not applied after June 14, except to the cultures lacking manganese.

The data were analyzed by the use of analysis of variance, and adjusted for variations in original weight by the use of analysis of covariance.

RESULTS

GROWTH IN SAND AND IN PEAT-ON-SAND MEDIA

With full nutrients, the blueberry plants set in sand grew rapidly and developed vigorous new growth and large green leaves similar to those of plants grown under optimum outdoor conditions (figs. 1 to 3). However, the plants grown in peat-on-sand were even more vigorous (fig. 4), and this was true both for those that received the full-nutrient solution and those deprived of one element, except potassium. As detailed later, nutrient deficiencies of certain elements did not appear as early in the peat-on-sand cultures as in sand cultures, and in the peat-on-sand cultures without calcium and without sulfur no deficiency symptoms occurred during the course of the experiments. The addition of growth substances had no effect on these results.

DEFICIENCY SYMPTOMS (pl. 1)

In the following discussion of the symptoms caused by a deficiency of the various mineral elements, the chronological appearance of symptoms is given as well as detailed aspects and comparisons. When there were no definite differences in the time that symptoms appeared in the two media, no discussion of media is included.

Symptoms of nitrogen deficiency (pl. 1, *A*) appeared during the first week of April, only 10 days after the buds began to leaf out. By April 30, the plants were so severely affected that small quantities of calcium nitrate had to be added to keep them alive. The early symptom of uniform yellowing of the entire leaf surface was followed by a reddening of the leaves, and dying. All the leaves were affected. The entire plants, tops and roots, were severely stunted.

Potassium deficiency (pl. 1, *B*) next became evident, being readily discernible by April 20. The sequence of symptom expression was

[5] Analysis of the tap water showed the following in parts per million: Silicon dioxide, 12; iron, 0.02; calcium, 8.6; magnesium, 2.3; sodium, 4.4; potassium, 1.5; carbonic acid, 23; sulfate, 15; chloride, 4.8; nitrate, 1.6. Total dissolved solids, 60.

complicated by the periodic "abortion" of the terminal growing points, a phenomenon which seems to be characteristic of most species of *Vaccinium* in the field as well as in the greenhouse. Consequently,

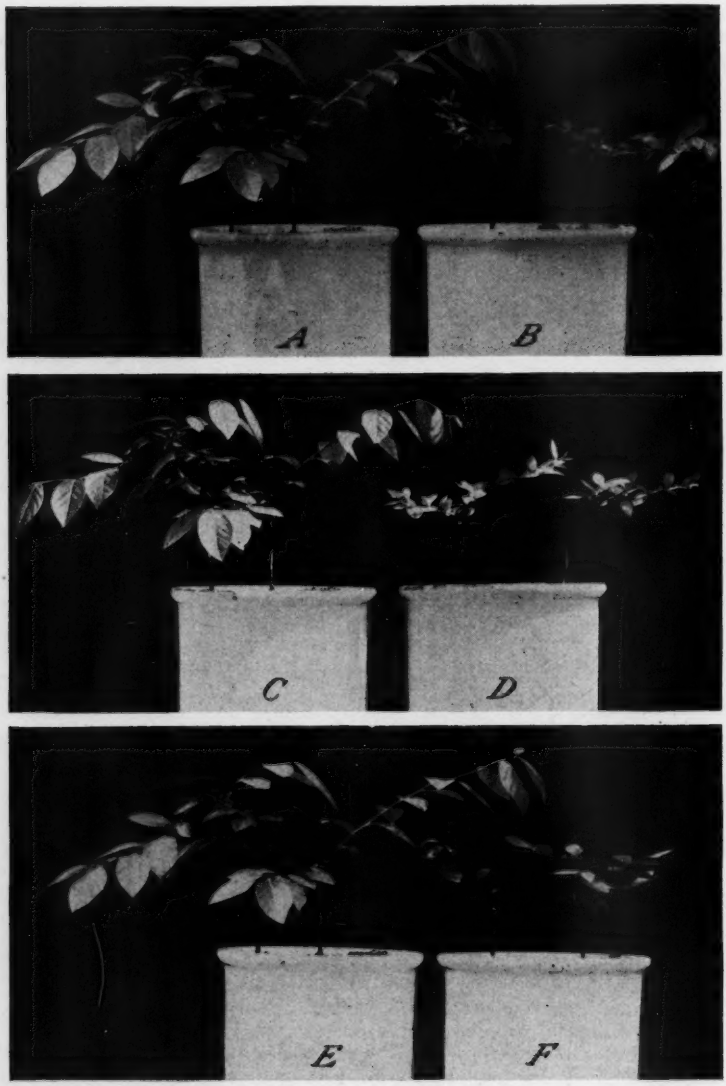


FIGURE 1.—The effect of certain mineral-nutrient deficiencies on the growth of the Cabot blueberry, photographed May 27, 1940: *A*, *C*, and *E*, Full-nutrient solution; *B*, nitrogen omitted; *D*, sulfur omitted; *F*, phosphorus omitted.

the more severe symptoms of marginal scorching and development of necrotic spots throughout the leaves appeared first on the older leaves, while interveinal chlorosis, similar to what has been described as iron

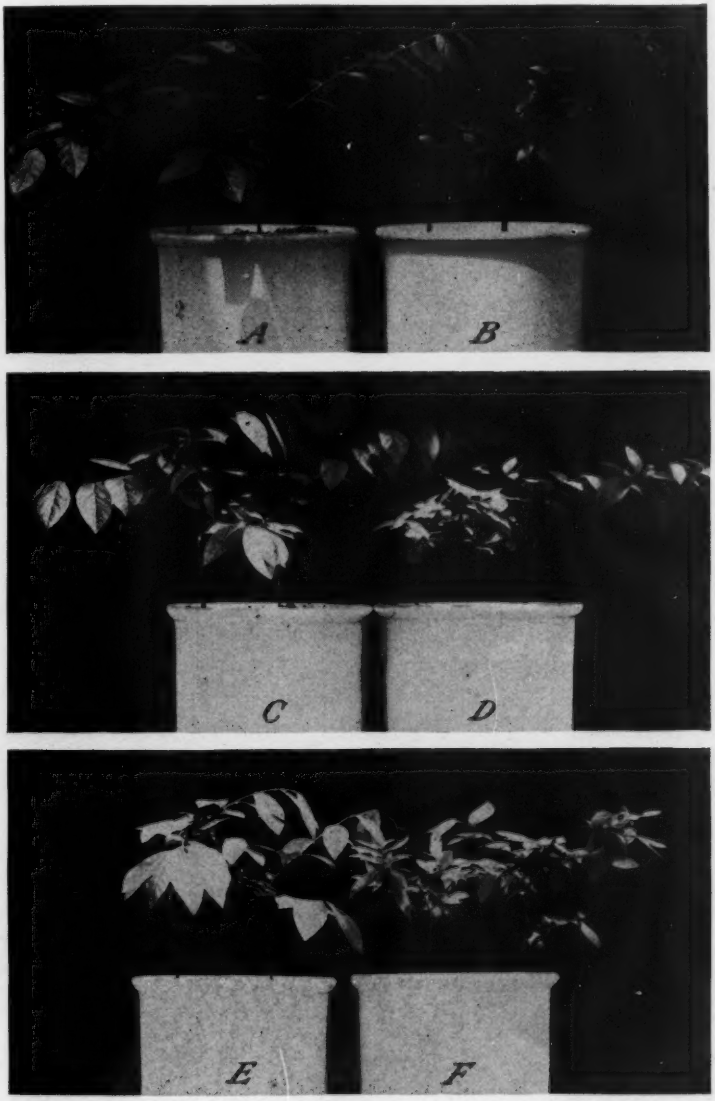


FIGURE 2.—The effect of certain mineral-nutrient deficiencies on the growth of the Cabot blueberry, photographed May 27, 1940: *A*, *C*, and *E*, Full-nutrient solution; *B*, boron omitted; *D*, potassium omitted; *F*, calcium omitted.

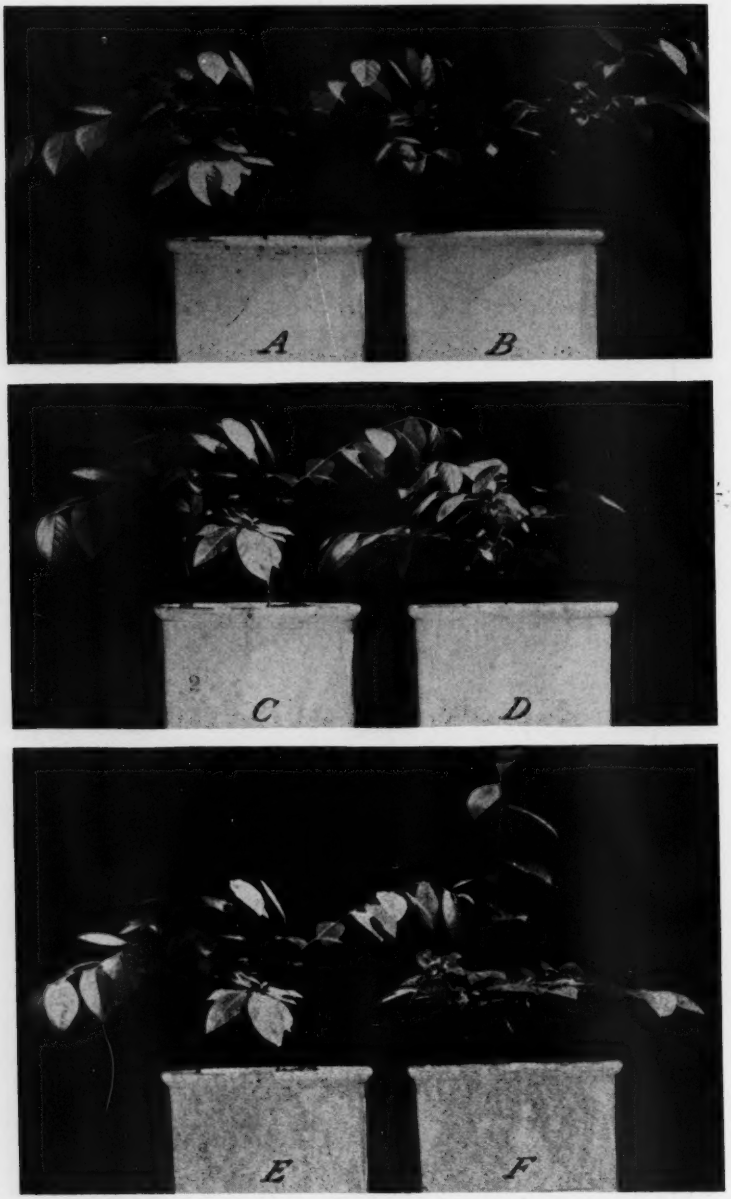


FIGURE 3.— The effect of certain mineral-nutrient deficiencies on the growth of the Cabot blueberry, photographed May 27, 1940: *A*, *C*, and *E*, Full-nutrient solution; *B*, magnesium omitted; *D*, iron omitted; *F*, manganese omitted.

chlorosis (*1*), appeared later on the new growth arising from axillary buds stimulated into growth after the terminal growing point aborted.

FIGURE 4.— Influence of the medium upon the development of mineral-nutrient deficiency symptoms in the Cabot blueberry: *A*, Boron omitted, peat-on-sand; *B*, boron omitted, sand; *C*, sulfur omitted, peat-on-sand; *D*, sulfur omitted, sand; *E*, calcium omitted, peat-on-sand; *F*, calcium omitted, sand.

Sulfur deficiency (pl. 1, *C*) was apparent only in the pure sand cultures. The symptoms were similar to those caused by nitrogen deficiency in the early stages, except that the yellowing had a more

bleached appearance. Whereas the plants lacking nitrogen turned red at a later stage, those lacking sulphur turned a faint pink. The older leaves retained their green coloration.

Calcium-deficiency symptoms (pl. 1, *D*) also appeared in the sand cultures only. The leaf symptoms were similar to those induced by potassium deficiency on the younger growth (iron deficiency?), but the regions adjacent to the veins that remained green were considerably narrower. The veins proper remained green, in contrast to those of the plants lacking sulfur. In advanced stages it was difficult to distinguish between the leaf symptoms of calcium sulfur deficiencies. Both tops and roots of the calcium-deficient plants were less severely stunted than the sulfur-deficient plants.

Boron-deficiency symptoms (pl. 1, *E*) appeared with astonishing abruptness. On May 8, all plants appeared to be healthy, whereas on the following day all those growing in sand were decidedly affected. Plants growing in peat showed no deficiency symptoms until June 7. The first evidence of boron deficiency was a bluish color of the terminal growing point, followed by chlorotic spotting of the young leaves directly below the terminal. As the deficiency continued, the young leaves became badly blotched and misshapen.

The necrosis of the terminal growing points caused by boron deficiency apparently stimulated the axillary buds into growth, and the symptoms first evidenced at the terminal growing points were repeated at each axillary growing point. These symptoms are easily distinguished from the natural abortion of the terminals, since the latter is definitely limited to the growing point, and the young leaves remain entirely normal; moreover, the discoloration of the naturally occurring abortion is brown, not blue.

Magnesium deficiency (pl. 1, *F*) did not cause as severe dwarfing as phosphorous deficiency, but by May 15 it had produced one very definite symptom. The leaf margins had become uniformly chlorotic while the leaf area near the midrib retained its normal green color. At a later stage, the chlorotic area became red and necrotic, while the rest of the leaf remained green. The older leaves were affected.

Phosphorous-deficiency symptoms (pl. 1, *G*) were not readily discernible until June 5, when they appeared as a slight purpling of the leaves and stems. Dwarfing of the plants, however, was noticeable as early as May 5. The color of the leaves was decidedly dull, in contrast to the bright glossy color of healthy blueberry leaves.

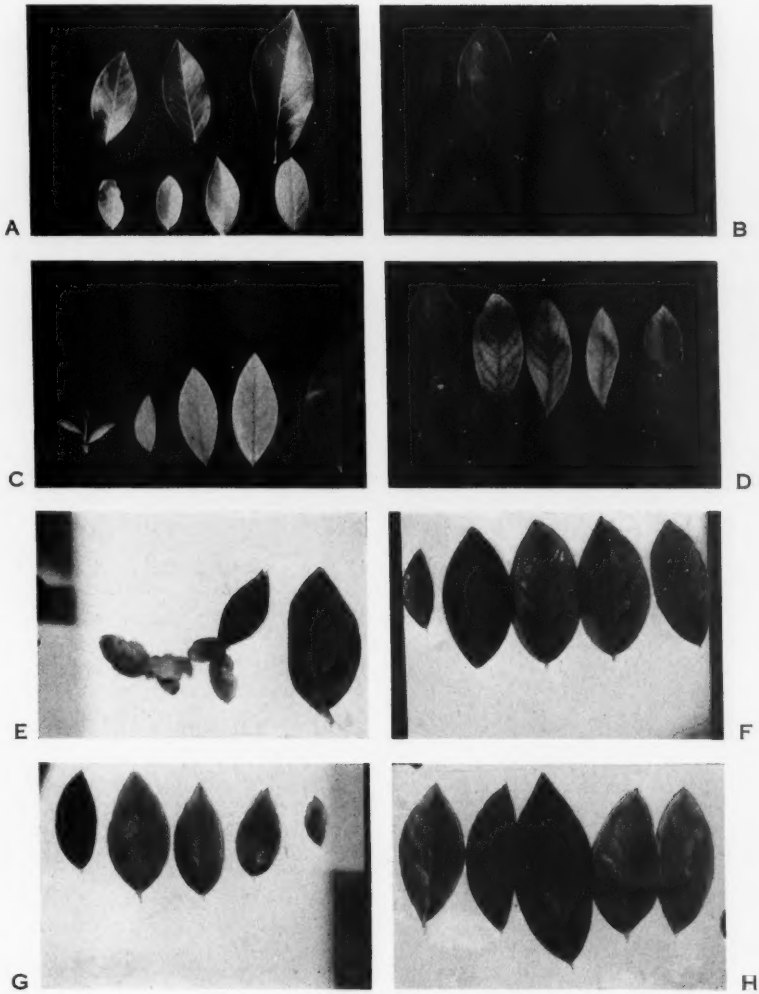
Iron deficiency (pl. 1, *H*), contrary to expectation, appeared as late as May 15, and then in the sand cultures only, as interveinal chlorosis of the younger leaves, very similar to, but less severe than, the potassium deficiency symptoms on the younger leaves.

Manganese deficiency did not become clearly apparent during the experimental period, although some manganese-deficient cultures were continued until the end of July.[6] There was some indication that lack of manganese resulted in a break-down of the axillary buds.

GROWTH SUBSTANCES

It has often been suggested that there is some substance or substances in peat that stimulate plant growth. If such is the case,

[6] The nutrient solutions were made with tap water after June 15



Leaf deficiency symptoms of the Cabot blueberry when grown in sand cultures. Leaf symptoms are shown in order of chronological appearance of the deficiencies, as follows: *(A)* nitrogen deficiency, lower row of leaves, compared with full nutrient, upper row of leaves; *(B)* potassium deficiency; *(C)* sulfur deficiency; *(D)* calcium deficiency; *(E)* boron deficiency; *(F)* magnesium deficiency; *(G)* phosphorus deficiency; *(H)* iron deficiency.

then the substance is probably not thiourea, vitamin B_1, or a substance of similar function and mechanism, since the addition of these substances had no significant effect on plant growth. Eight other substances, indoleproprionic acid, indoleacetic acid, Photo Sen Sen, indole-acetamide, alpha-naphthylacetamide, indolebutyric acid, alpha-naphthaleneacetic acid, and ascorbic acid, reportedly capable of stimulating plant growth, had no significant effect when applied in various combinations and concentrations to blueberry plants growing in silt loam.

FRESH-WEIGHT DETERMINATIONS

Inasmuch as growth substances had no significant effect on the plants, the six plants of each nutrient treatment for each medium that received these substances were included in studies of fresh and dry weight as affected by nutrients and media only.

The fresh weights of the plants removed after 6 weeks' growth are given in table 2. At this early stage in the peat-on-sand medium, only plants deficient in nitrogen, potassium, and phosphorus showed a significantly lower top weight, and only plants deficient in potassium and nitrogen showed a significantly lower root weight than the plants receiving the full-nutrient solution. In the sand medium, the top weights of the calcium- and nitrogen-deficient plants were significantly low, and only the nitrogen-deficient plants had a significantly lower root weight than the full-nutrient plants.

At this time there was no significant difference in the top growth of the plants in the two media, but there was already a highly significant difference favoring the peat-on-sand medium in respect to root growth. The sulfur-deficient plants especially made better root growth in the peat-on-sand medium than in the sand alone.

The F value of 2.61 for growth substances was not quite high enough to be significant at the 5 percent level. The size of the value that was obtained was apparently chiefly due to an inhibition of top growth by the application of thiourea, and a possible improvement of the calcium-deficient condition in the sand medium by the addition of thiamin chloride.

By June 10–14, after 12 weeks of growth, additional differences became apparent (fig. 5, and table 2). At this time the factor of media was responsible for very significant differences in both top and root weights, as indicated by the values of F for media in table 2. All but the potassium-deficient plants made better growth in peat-on-sand than in sand, and the significance of these differences is supported by the significant interaction, nutrients $\times$ media.

Considering the plants as a whole, the various deficiencies as they appeared after 12 weeks of growth may be grouped broadly into three categories according to total weight of plants: (1) Those that were not poorer than the plants receiving the full-nutrient solution[7]; (2) those poorer but yet making some growth; and (3) those so severely affected that they could make no further growth unless the deficient element was supplied promptly. Of the plants growing in the peat-on-sand

[7] Although the plants deficient in the elements listed in group 1 did not have a significantly lower weight than the plants receiving all the nutrients, all but the manganese-deficient plants exhibited definite deficiency symptoms (cf. p. 320). It should be noted that in the sand medium, only the anion deficiencies caused a severe reduction in growth.

medium, those receiving a nutrient solution deficient in manganese, iron, sulfur, and calcium fall in the first group; those deficient in boron and magnesium in the second; and those deficient in phosphorus, potassium, and nitrogen in the third. In the sand medium, the plants deficient in potassium and magnesium move up into the first group, those deficient in sulfur and boron drop to the last group, and those deficient in calcium remain alone in the middle group.

TABLE 2.—*Fresh weight of Cabot blueberry plants (average of six plants) grown in peat-on-sand and in sand with full-nutrient solution and with deficiencies in certain mineral nutrients, and removed from culture between April 29 and May 1 and between June 10 and 14, 1940* [1]

PLANTS REMOVED APRIL 29 TO MAY 1, 1940

Mineral deficiency	Current top growth		Root growth [2]		Total growth		Shoot-root ratio [2]	
	Peat on sand	Sand	Peat on sand	Sand	Peat on sand	Sand	Peat on sand	Sand
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*		
None	2.43	2.17	10.59	8.32	13.02	10.49	0.23	0.26
Manganese	2.14	1.96	9.41	7.41	11.55	8.37	.23	.26
Iron	2.40	2.02	7.70	8.06	10.10	10.08	.31	.25
Sulfur	1.92	1.71	12.61	7.98	14.53	9.69	.15	.21
Calcium	1.96	1.45	10.88	10.06	12.84	11.51	.18	.14
Boron	2.27	2.15	9.61	8.76	11.88	10.91	.24	.25
Magnesium	1.96	2.04	10.59	8.57	12.55	10.61	.19	.24
Phosphorus	1.67	1.65	8.75	7.63	10.42	9.28	.19	.22
Potassium	1.61	2.25	6.51	7.57	8.12	9.82	.25	.30
Nitrogen	1.06	.89	5.56	5.59	6.62	6.48	.19	.16
Difference required for significance [3]	0.64		2.95					
F values [4] for:								
Nutrients	5.36**		4.03**					
Media	.056		7.08**					
Growth substances [5]	2.61		.78					
Nutrients × media	1.32		1.03					

PLANTS REMOVED JUNE 10–14 [1]

None	12.33	8.63	22.13	14.38	34.46	23.01	0.56	0.60
Manganese	15.07	7.71	18.02	11.90	33.09	19.61	.84	.65
Iron	16.53	6.48	15.73	11.82	32.26	18.30	1.05	.55
Sulfur	10.41	3.41	20.16	9.24	30.57	12.65	.52	.37
Calcium	14.38	6.73	15.16	9.98	29.54	16.71	.95	.67
Boron	14.09	3.27	13.82	10.16	27.91	13.43	1.02	.32
Magnesium	11.85	6.13	12.87	13.60	24.72	19.73	.92	.45
Phosphorus	5.50	2.86	11.38	10.07	16.88	12.93	.48	.28
Potassium	5.27	6.82	8.10	11.50	13.37	18.32	.65	.59
Nitrogen [6]	4.01	1.94	7.97	7.01	11.98	8.95	.50	.28
Difference required for significance [3]	3.64		5.54					
F values [4] for:								
Nutrients	12.28**		4.73**					
Media	87.45**		16.64**					
Growth substances [5]	.15		.11					
Nutrients × media	4.31**		2.32*					

[1] All values adjusted for variations in original weight of cuttings by the use of analysis of covariance.
[2] Entire weight, minus the current top growth, included in "Root growth."
[3] Upon the suggestion of Dr. A. E. Brandt, of the Soil Conservation Service, U. S. Department of Agriculture, the accuracy of this value was tested by analyzing separately the data of individual deficiency treatments against the data of the full-nutrient treatment. Of the treatments tested by this more precise method, no additional significant values were obtained, and the values found to be significant by the more general method remained significant when treated by the more precise method.
[4] **Significant beyond the 1-percent point; *significant beyond the 5-percent point.
[5] Values for growth substances are not given, since they caused no significant differences when the data were treated by the more general and the more precise methods of analysis.
[6] Calcium nitrate added in small quantities from April 30, on.

When top and root growth are considered separately, the results vary considerably (table 2). It appears that when manganese, iron, calcium, or boron was withheld from blueberry plants growing in the peat-on-sand medium, those plants actually made greater top growth

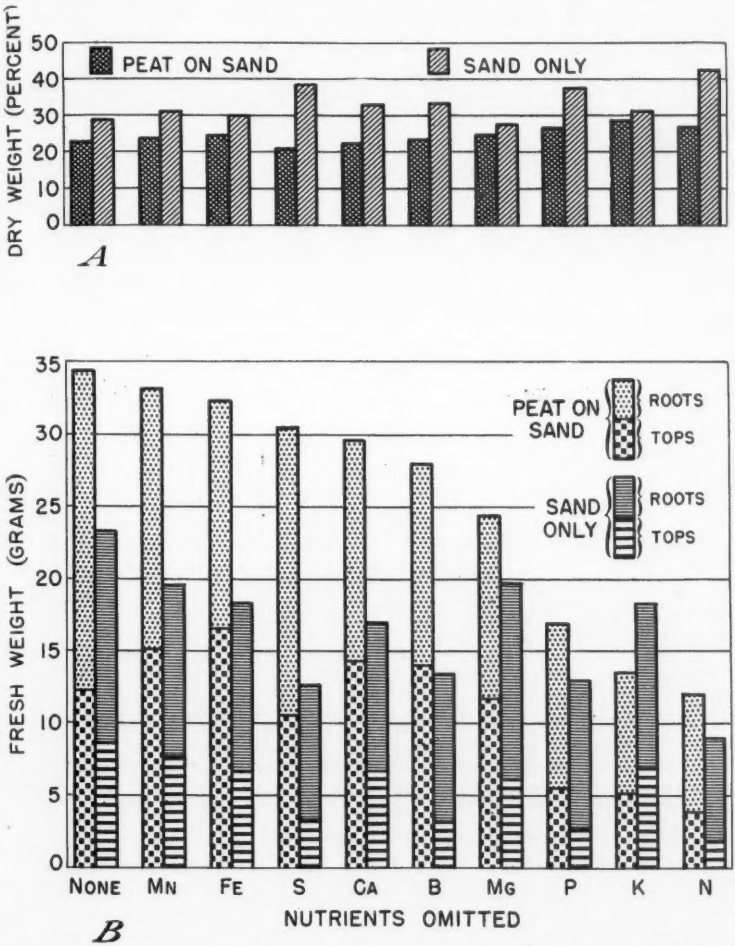


FIGURE 5.—Average dry weight (*A*) and fresh weight (*B*) of rooted blueberry cuttings after 12 weeks of growth in peat-on-sand and in sand, with full-nutrient solution and with solutions from which certain mineral nutrients were omitted.

than plants receiving the full nutrient solution. The iron-deficient plants made a significantly greater top growth, while the others approached a significantly higher difference. Phosphorus, potassium, or nitrogen deficiency, on the other hand, resulted in a significant decrease in top weight. In the sand medium, the full-nutrient plants

made the greatest top growth; top growth of the nitrogen-, phosphorus-, sulfur-, and boron-deficient plants was significantly low. As for root weights, the full-nutrient plants—both those grown in peat-on-sand and those grown in sand—showed the greatest weight, being significantly higher than all but the manganese- and sulfur-deficient plants in peat-on-sand, but significantly higher than only the nitrogen-deficient plants in sand.

TABLE 3.—*Dry weight of Cabot blueberry plants grown in peat-on-sand and in sand with full-nutrient solution and with deficiencies in certain mineral nutrients, and removed from culture April 29–30 and June 10–14, 1940*

Mineral deficiency	Dry weight Apr. 29–30				Dry weight June 10–14				Dry weight [1] of current top growth June 10–14	
	Current top growth		Total plant growth		Current top growth		Total plant growth			
	Peat on sand	Sand	Peat on sand	Sand	Peat on sand	Sand	Peat on sand	Sand	Peat on sand	Sand
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Grams*	*Grams*
None	31.0	34.3	28.5	41.7	35.5	39.2	22.5	29.3	4.27	3.24
Manganese	31.2	33.3	29.3	45.5	30.2	39.3	24.0	31.3	4.50	3.03
Iron	28.8	32.7	33.2	40.8	30.0	40.5	24.7	30.3	4.93	2.59
Sulfur	31.0	33.0	24.0	44.0	33.7	53.0	20.8	38.5	3.32	1.74
Calcium	30.5	29.3	29.5	38.8	29.8	42.7	22.8	33.2	4.25	2.58
Boron	29.3	32.8	29.0	35.8	31.0	48.8	23.5	33.5	3.98	1.64
Magnesium	31.8	32.0	27.8	41.3	31.7	39.7	25.2	27.5	3.70	3.06
Phosphorus	31.8	33.0	26.5	40.3	42.8	58.2	27.3	37.3	2.10	1.73
Potassium	31.5	32.7	31.2	42.0	39.7	42.0	28.7	31.2	2.23	2.34
Nitrogen	34.5	35.8	41.5	45.8	45.0	48.8	27.3	42.7	1.62	1.04
Difference required for significance	2.61		4.56		5.78		4.57		1.04	
F values [2] for:										
Nutrients	5.31**		6.03**		13.36**		5.93**		11.04**	
Media	18.67**		256.66**		126.37**		148.91**		51.68**	
Growth substances	1.93		.90		2.86**		.64		.22	
Nutrients×media	1.48		4.65**		4.33**		4.75**		2.45*	

[1] Data adjusted for variations in original weight of cuttings by use of analysis of covariance.
[2] **Significant beyond the 1-percent point. *Significant beyond the 5-percent point.

These variations in shoot and root response are also expressed in terms of shoot-root ratios (table 2). Thus, after 12 weeks' growth (June 10–14) the manganese-, iron-, calcium-, boron-, and magnesium-deficient plants growing in peat-on-sand had a much higher ratio than the full-nutrient-grown plants, while the sulfur-, boron-, phosphorus-, and nitrogen-deficient plants growing in sand had a markedly lower ratio than the plants receiving the full-nutrient solution.

DRY-WEIGHT DETERMINATIONS

The percentage of dry weight of roots, tops, or total plants was not greatly influenced by the various nutrients during the first 6 weeks' growth (table 3). Only the nitrogen-deficient plants in peat-on-sand, which were already severely affected, had a significantly higher percentage of dry matter than the full-nutrient treatment.

Considering all treatments, the average dry weight of the top growth was about 31 percent in the peat-on-sand, and 33 percent in the sand medium. The entire plants averaged about 30 percent in the peat-on-sand medium, and 42 percent in the sand. In the peat-on-sand

medium the tops had a greater percentage of dry weight than the roots, while in the sand medium the roots had the greater percentage of dry weight.

After 12 weeks' growth it was clearly apparent that those plants that had the greater fresh weight also had the smaller percentage of dry weight (fig. 5). When calculated, this relationship resulted in a negative correlation of −0.71. Such a highly significant correlation indicated the possibility that some of the differences in fresh weight given in table 2 might be due to variations in moisture content alone. However, the dry-weight data showed essentially the same relative differences as the fresh-weight data (cf. tables 2 and 3), except that the differences between the treatments were greater on a fresh-weight basis, and therefore more apparent.

ABORTION OF THE TERMINAL GROWING POINTS

The percentage of terminal growing points aborted by April 7, or 3 weeks after planting, was determined. A significantly greater percentage of growing points aborted in the plants from which nitrogen was withheld than in the other plants. Sixty percent of the terminals of the minus-nitrogen plants in peat-on-sand and 67 percent of those in sand aborted, as compared with 25 to 51 percent on other treatments, and an average of 35 percent in peat-on-sand and 33 percent in sand. The correlation between top weight and percent of terminals aborted was found to be −0. 71 for the plants growing in sand, −0. 47 for the plants growing in peat-on-sand, and −0. 60 for all plants regardless of media. All the correlations are significant.

DISCUSSION

NUTRIENT DEFICIENCIES

If it may be said at all that one element limits growth more than another, then it is obvious that nitrogen is the limiting element in the normal growth of blueberry plants. Such a conclusion is supported by considerable field data which serves as a basis for the recommendation of fertilizers high in nitrogen (*1*, *2*, *3*), and it is further supported by the observations of Doehlert and Shive (*5*) and Stene (*13*) made on plants grown in the greenhouse.

Assuming that the sand medium approaches the physical and chemical condition of a poor sandy soil, the various elements in such a soil might be expected to become deficient for growth of the blueberry in the following order: Nitrogen, phosphorus, sulfur, boron, calcium, potassium, iron, magnesium, and manganese. In like manner, if the peat-on-sand medium may serve as an example of a soil high in organic matter, the order of importance in such a soil might be expected to be: Nitrogen, potassium, phosphorus, magnesium, boron, calcium, sulfur, iron, and manganese.

Earliness of symptom appearance is not necessarily related to the degree of injury or stunting of growth. Potassium-deficiency symptoms, for example, appeared sooner than any other except nitrogen symptoms in both media, and yet the total plant weight in the sand medium after 12 weeks' growth still compared favorably with that of magnesium and iron-deficient plants, which showed deficiency symptoms much later.

As already mentioned, the milder symptom of potassium deficiency was very similar to the condition previously reported as iron chlorosis (*1*). Apparently, a lack of potassium results in making iron unavailabile. Hoffer (*9*) made use of this observation in testing corn for potassium deficiency, a precipitation of iron in the nodes indicating a low level of potassium. A further indication that the above symptom is brought about by the unavailability of iron was provided by an experiment with blueberry plants grown in the greenhouse during the summer and subjected to temperatures as high as 120° F. and high light intensities. These plants showed mild symptoms of potassium deficiency when grown in sand, but almost none when grown in peat. Plants grown in silt loam were also affected, but not so severely as the plants grown in sand. pH determinations were made with a glass electrode on material that exhibited various degrees of chlorosis. The pH reading of sap from normally green tissue was 3.3, and increased with increasing severity of the chlorosis to pH 4.2.

Since Gericke (*7*) showed that high light intensity was detrimental if iron was not sufficiently available, and Ingalls and Shive (*10*) demonstrated that the acidity of plant sap decreases with increased light intensity, it is possible that the high light intensity of summer days decreases the acidity of the blueberry plant sap to such a degree that the iron which was available in the nutrient solution or in the soil solution, becomes unavailable in the plant sap.

That the symptom is not a simple effect of the hydrogen-ion concentration of the nutrient medium is shown by the fact that those plants grown in soil having a pH value of 4.4, considered optimum for blueberry growth (*12*), were also affected.

MEDIA

All but the potassium-deficient plants made better growth in the peat-on-sand medium than in sand alone. Possibly the absence of potassium in the peat caused other materials as well to be made unavailable (iron?), or actually toxic.

The generally beneficial effect of peat can be partly attributed to the presence in available form of sulfur, calcium, and boron, since plants not receiving any one of these elements grew normally in peat-on-sand, and very poorly in sand alone. There is, however, an additional benefit derived from peat, as indicated by the significantly higher weights of the full-nutrient plants growing in peat-on-sand over those in sand. It may be that the peat provides the plant with a more uniform and cooler temperature, and a greater supply of moisture. The peat may also have an indirect effect on the hydrogen-ion concentration of the plant sap.

SHOOT-ROOT RATIOS

The significant effect of peat on the shoot-root ratio is not unusual, for similar results have been reported on other crops. Havis (*8*), for example, working with the black raspberry, found more root and less cane growth under cultivation than under mulch. The notable effect of nutrient deficiencies on the shoot-root ratio showed that upon omission of manganese, iron, calcium, boron, or magnesium from the peat-sand medium, the tops were larger in proportion to the roots, as compared to full-nutrient plants. It may be that the above deficiencies

affect the roots more severely than the shoots. It is evident, however, that boron deficiency affects the shoot tip more than any other plant organ. The elements were apparently not present in too high concentrations, since the total weight of the full-nutrient plants was the highest.

SUMMARY

Rooted cuttings of the Cabot blueberry were grown in peat-on-sand and sand media, and supplied with various nutrient solutions and growth substances.

In the peat-on-sand medium no symptoms of calcium, iron, or sulfur deficiency were observed during the course of the experiments, and symptoms of boron deficiency were late in appearing. In the sand medium, iron-deficiency symptoms also were late in developing. With these exceptions deficiency symptoms appeared after different lengths of time in both media in the following order: Nitrogen, potassium, sulfur, calcium, boron, magnesium, phosphorus, iron, and manganese.

The beneficial effect of peat was partly due to the presence of calcium, sulfur, and boron in available form, and partly to factors that could not be determined, since plants receiving the full-nutrient solution also made better growth, although to a lesser degree, in the peat-on-sand medium than in sand alone.

The characteristic deficiency symptoms for each element were found to be sufficiently unlike to be easily identified, particularly those caused by nitrogen, potassium, boron, sulfur, magnesium, and phosphorus.

The shoot-root ratio of plants growing in the peat-on-sand medium was greatly increased when manganese, iron, calcium, boron, or magnesium was omitted from the nutrient solution, and decreased when sulfur, boron, phosphorus, or nitrogen was withheld from the sand-grown plants.

There was a significant negative correlation between percentage of fresh weight and percentage of dry weight, but the results based on dry weight were practically identical with those obtained with fresh weights, since the differences in weight were sufficiently large to be maintained on a dry-weight basis also.

Lack of nitrogen significantly increased the rapidity with which terminal growing points aborted. There was a significant correlation between percentage of terminals aborted and top weight.

Weekly applications of thiourea and vitamin B_1 had no significant effect on the deficiency symptoms, fresh or dry weights of the plants, or growing-point abortion. Eight other substances applied to blueberry plants growing in silt loam soil also had no significant effect.

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SURVIVAL OF SHEEP NEMATODES IN PASTURES[1]

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INTRODUCTION

Pasture rotation is one of the most important measures recommended for the control of gastrointestinal parasites of sheep. Since the acquisition of these parasites by grazing sheep depends on the presence and availability of infective eggs and larvae, recommendations for pasture rotation must be based on a knowledge of the longevity of these infective agents on pastures. The available information on this subject has been obtained largely from laboratory studies. Pasture experiments in which parasite-free sheep were used to test the survival periods of infective eggs and larvae under natural conditions are comparatively few in number, so far as published records show. Consequently, such experiments were carried on in 1938 and 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. The results of this work are reported in the present paper.

REVIEW OF LITERATURE

Ever since Ransom (*5*)[2] demonstrated that ensheathed larvae of the common ruminant stomach worm, *Haemonchus contortus*, not only survived from December 27 to March 22, but also showed no appreciable reduction in the number of contained food granules, even though these larvae were in a frozen condition almost one-fourth of the time, it has been generally accepted that they are able to survive the winter months. This view was supported by the work of Dikmans and Andrews (*2*), of Griffiths (*3*), and of Baker (*1*). On the other hand, Zavadovsky and Vorobiova (*8*) reported that "Trichostrongylidae larvae, spending winter in the open under Moscow climatical conditions, are practically deprived of any invasive significance towards spring," and Schmid (*6*) reported that in Germany sufficient pasture infection does not last over winter to cause a massive invasion of the host animals.

Until recently, however, studies of survival, during the grazing season, of preinfective stages of ruminant nematodes have been neglected for the most part.

Leiper[3] suggested that sheep should do well during a dry summer because of the destructive effect of sunlight on the early stages of those ovine nematodes that have thin-shelled eggs. In studies on the eggs and larvae of *Haemonchus contortus* and *Trichostrongylus* spp., Kauzal in Australia, as reported by Julius and coworkers (*4, p. 33*), found that when pastures were left unstocked for 1 to 2 months during

[1] Received for publication March 12, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 336.

[3] LEIPER, R. T. THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE SPREAD OF PARASITIC WORMS. Agr. Met. Conf. (So. Kensington, England, 1928) Rpt., pp. 79–82. [1929] [Processed.]

periods of continuous dry weather, the degree of infection with these nematodes was greatly reduced. In *H. contortus*, he thought that complete eradication might be effected in this manner. Taylor (7) stated that there was a rapid dying off of infective trichostrongyle larvae kept on grass in a box in the laboratory, as compared with larvae on grass placed in a box in the field. The longest period that he found larvae to survive in the field was 133 days. He regarded it as probable that the longest-lived larvae were those that remained in the "moistest microclimate" on the herbage nearest the soil, and that some might remain actually in the soil temporarily and migrate to the herbage some time later. The experiments of Taylor and those of Kauzal showed that in England and Australia, respectively, the larvae of trichostrongylid nematodes did not survive long under dry summer conditions.

Of the experiments mentioned, only those of Dikmans and Andrews, of Griffiths, and of Baker actually involved testing the survival of infective stages by grazing sheep on previously contaminated pastures.

MATERIALS AND METHODS

Of the five experiments reported in this paper, the first three were made on summer pastures. The pasture used in experiment 1 had a heavy grass cover with little shade; in experiment 2, little grass and little shade; and in experiment 3, abundant grass and rather dense shade. Experiment 4 was made in the late fall and winter on the pasture used in experiment 1; experiment 5, in the spring on the pasture used in experiment 3. The three pastures consisted of level, one-fourth-acre areas that, so far as is known, had not been previously occupied by sheep.

In order to contaminate these pastures, infected ewes were permitted to graze on them for 2 weeks and were then removed. Lambs free of nematodes except *Strongyloides* were then allowed to graze on a part of or the entire pasture for 2 weeks. The presence of *Strongyloides* in the lambs was due to the fact that these parasites cannot be controlled by means used to control other nematodes. At the end of 2 weeks the lambs were removed to concrete-floored outdoor pens, where they were kept for a sufficient time to permit the infective larvae or eggs that might have been ingested to develop into mature worms.

To determine the species of nematodes in the sheep that were used to contaminate the pastures and also the species that had been acquired by the lambs while on the contaminated areas, the following procedure was used. In the first experiment, post mortem examinations were made on the sheep and lambs; in the other experiments examinations of the feces were made by the direct centrifugal flotation method. Differential egg counts were made on one pellet from each animal for each examination. In the lambs, the fecal examination was supplemented by culturing 50 gm. of feces from each animal and examining these cultures for the presence of larvae. The use of the fecal examination in all experiments after the first was due to the perfecting of methods of differentiating eggs of nematodes in sheep, thus making it unnecessary to destroy the animal in order to determine what nematodes were present.

Fecal examinations were made before the animals were placed on the pasture, while they were on it, and just after they had been removed, to be sure that they had no nematodes other than *Strongyloides*. None of the lambs were found to be infected. If no eggs were found in fecal examinations of lambs after they had been kept in the pens for the allotted time, cultures were made as a more exact diagnostic method of determining presence or absence of infection. If infective larvae were present on the pasture, the period of 2 weeks during which the lambs remained there was adequate to permit infection but too short to permit such larvae to grow into adult worms. Further infection of the pasture by the lambs was thus avoided.

WEATHER DURING THE EXPERIMENTS

The survival of infective larvae, as well as the development of preinfective larvae, of sheep nematodes depends to a large extent on the weather. For this reason, table 1, which shows the weather conditions that prevailed during the experiments, is included. These data were collected by the United States Weather Bureau in Washington, D. C., about 20 miles from the location of the experimental pastures. The temperatures at Washington are usually 1° to 6° F. warmer than those at the experiment station. Humidity and rainfall differ only slightly and total sunshine is about the same in the two localities.

TABLE 1.—*Summary of weather data collected at Washington, D. C. (about 20 miles from the experimental pastures) during the time of the experiments*

Month	Temperature			Precipitation		Average sunshine	Average relative humidity at—	
	Mean	Maximum	Minimum	Total	Days		7:30 a. m.	12 m.
1938	° *F.*	° *F.*	° *F.*	*Inches*	*Number*	*Percent*	*Percent*	*Percent*
June	72.8	95	45	2.26	22	59	77	51
July	69.1	96	60	5.06	20	56	81	57
August	78.6	96	60	4.64	10	75	78	54
September	67.4	90	48	4.27	16	39	82	63
October	58.8	88	38	1.15	7	73	82	52
November	49.8	79	14	3.60	13	64	82	57
December	38.4	59	20	2.69	15	41	72	58
1939								
January	37.8	63	17	3.41	17	43	72	55
February	42.3	74	17	5.71	15	43	71	52
March	46.4	85	26	2.89	17	50	68	49
April	53.4	90	31	3.78	20	47	68	50
May	68.0	95	40	.41	9	69	70	44
June	75.4	96	59	4.55	17	55	75	59
July	76.2	93	58	2.01	20	55	78	57
August	78.8	96	64	3.22	14	56	78	51
September	71.0	100	51	6.90	11	56	85	53
October	58.4	92	35	4.06	14	56	81	54
November	46.0	72	30	1.40	11	61	69	41

SURVIVAL OF NEMATODES ON VARIOUS TYPES OF SUMMER PASTURES

EXPERIMENT 1 (HEAVY GRASS COVER WITH LITTLE SHADE)

The first pasture, which had a rather heavy grass cover but little shade, was grazed from June 20 to July 5, 1938, by six heavily parasitized ewes. After this period of grazing the ewes were killed and examined. The species of nematodes and the number of each found in the ewes are given in table 2.

TABLE 2.—*Species and number of nematodes found, on post mortem, in 6 ewes grazed from June 20 to July 5, 1938, on a pasture with a rather heavy cover of grass but little shade* [1]

Nematode [2]	Nematodes in ewe No.—						Total nematodes
	1	2	3	4	5	6	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Bunostomum trigonocephalum	70	7	0	2	2	0	81
Capillaria brevipes	0	0	1	0	0	0	1
Chabertia ovina	3	0	0	4	3	4	14
Cooperia curticei	832	2,739	360	5,585	630	1,090	11,236
Cooperia oncophora	0	913	0	0	630	0	1,543
Haemonchus contortus	4,100	2,418	7,300	16,000	1,845	20,182	51,845
Nematodirus filicollis	104	0	435	0	210	218	967
Nematodirus spathiger	0	5,478	765	1,100	420	872	8,635
Oesophagostomum columbianum	75	5	4	5	7	17	113
Ostertagia circumcincta	300	124	1,158	6,400	345	356	8,683
Ostertagia trifurcata	150	0	0	0	116	0	266
Strongyloides papillosus	0	2,739	360	5,585	105	763	9,552
Trichostrongylus axei	1,350	31	0	800	790	1,246	4,217
Trichostrongylus colubriformis	0	4,565	425	4,468	1,050	1,090	11,598
Trichostrongylus vitrinus	312	3,652	2,550	7,819	5,670	872	20,875
Trichuris ovis	0	1	1	0	0	2	4

[1] In addition to the nematodes, the following numbers of the tapeworm *Moniezia expansa* were found: Five in ewe 2, seven in ewe 5, and four in ewe 6.

[2] A few specimens of *Dictyocaulus filaria* were found in ewes 2, 3, 4, and 5, and many were found in ewes 1 and 6. Because of the location of these parasites, counting was impracticable.

TABLE 3.—*Nematodes recovered, on post mortem, from lambs after 2 weeks of grazing on pasture previously contaminated by feces of ewes included in table 2*

Nematode [1]	Nematodes in lamb No.—			
	188 [2]	189 [2]	144 [3]	156 [3]
	Number	*Number*	*Number*	*Number*
Bunostomum trigonocephalum	0	0	0	0
Capillaria brevipes	0	0	0	0
Chabertia ovina	0	0	0	0
Cooperia curticei	124	164	0	0
Cooperia oncophora	0	0	0	0
Dictyocaulus filaria	0	0	0	0
Haemonchus contortus	6,814	4,648	0	0
Nematodirus filicollis	62	82	0	0
Nematodirus spathiger	248	738	0	0
Oesophagostomum columbianum	1	0	0	0
Ostertagia circumcincta	0	168	0	0
Ostertagia trifurcata	0	0	0	0
Strongyloides papillosus	589	205	0	0
Trichostrongylus axei	56	209	0	0
Trichostrongylus colubriformis	527	1,025	0	0
Trichostrongylus vitrinus	372	984	0	0
Trichuris ovis	0	1	3	1

[1] In addition to the nematodes, two specimens of the tapeworm *Moniezia expansa* were found in lamb 188, one specimen in lamb 189, and three in each of lambs 144 and 156.

[2] Placed on part of pasture 3 weeks after infected ewes were removed.

[3] Placed on second part of pasture 3½ months after infected ewes were removed.

Three weeks after the ewes had been removed, two 5-month-old lambs (Nos. 188 and 189) were placed on a one-seventh part of the pasture and allowed to graze for 2 weeks. Lamb 188 was found dead on the twenty-sixth day after it had been removed to the pen, and 4 days later lamb 189 was killed. Of the 17 species of nematodes originally present in the ewes, 11 were found in the lambs (table 3). The first lamb undoubtedly died of parasitism, but since death occurred on a holiday and the matter was not brought to the writer's attention when the dead animal was found, the post mortem examination was

not made until the next day. In the meantime some of the worms had probably disintegrated, since a large number of fragments of *Haemonchus contortus* were found. Even before death there must have been a loss of *H. contortus*, because many of these stomach worms were found in the small and large intestines.

On October 17, 1938 (about 3½ months after the ewes had been removed), two 7-month-old lambs (Nos. 144 and 156) were put on another one-seventh part of the pasture and removed to a pen on October 31. These lambs were killed 2 weeks later to prevent a possible loss of worms, suspected as having occurred in the previous test. The results, as shown in table 3, indicated that the preparasitic stages of the nematodes previously present on this pasture, except those of *Trichuris ovis*, had disappeared during the 3½-month period. This occurrence was so unexpected in the light of findings of previous investigators that the experiment, with certain modifications, was repeated in experiments 2 and 3.

EXPERIMENT 2 (PASTURE HAVING LITTLE GRASS AND LITTLE SHADE)

Twelve heavily infested ewes were allowed to remain, from May 15 to May 29, 1939, on a pasture with a sparse stand of grass and little shade.

Lambs 316 and 323, each 4 months old, were allowed to graze from June 13 to July 1, 1939, on a one-seventh part of this pasture. They became heavily infested with several kinds of nematodes, as shown in table 4.

TABLE 4.—*Nematode eggs found in feces of lambs after 2 weeks of grazing on previously contaminated pasture having little grass and little shade* [1]

Nematode	Nematode eggs in feces of lamb No.—			
	316 [2]	323 [2]	315 [3]	324 [4]
	Number	*Number*	*Number*	*Number*
Bunostomum trigonocephalum	0	3	0	0
Cooperia spp	12	143	0	0
Haemonchus contortus	1,525	932	0	0
Nematodirus spp	0	0	0	0
Oesophagostomum spp	5	1	0	0
Ostertagia spp	3	3	0	0
Trichostrongylus spp	22	84	0	0
Trichuris ovis	0	0	0	1

[1] Infection with the tapeworm *Moniezia expansa* was also found in lamb 315.
[2] Placed on part of pasture 2 weeks after infected ewes were removed.
[3] Placed on second part of pasture about 2½ months after infected ewes were removed.
[4] Placed on third part of pasture about 4½ months after infected ewes were removed.

Lamb 315, 5 months old, was allowed to graze on a similar part of this pasture from August 19 to September 2. No nematode eggs were found on subsequent fecal examinations. Lamb 324, 8 months old, was allowed to graze on a third part of the pasture from October 16 to October 30. No nematode eggs other than those of *Trichuris ovis* were found on subsequent fecal examinations.

In general the results of experiment 2 agree with those of experiment 1, as regards the period in which larvae of most species of nematodes disappear from the pasture. In this test the period was less than 2½ months.

EXPERIMENT 3 (SHADY PASTURE WITH ABUNDANT GRASS)

For the third experiment, a pasture with rather dense shade and lush grass was used. The 12 heavily parasitized sheep used in experiment 2 were placed on this plot and allowed to graze from May 29 to June 13, 1939.

On July 10, lambs 310 and 360, each 4 months old, were placed on one-sixth of this pasture and were removed on July 26. The results of differential egg counts, made on August 15, are shown in table 5.

TABLE 5.—*Nematode eggs found in feces of lambs after 2 weeks of grazing previously contaminated pasture having dense shade and abundant grass* [1]

Nematode	Nematode eggs in feces of lamb No.—			
	310 [2]	360 [2]	309 [3]	322 [4]
	Number	*Number*	*Number*	*Number*
Bunostomum trigonocephalum	0	1	0	0
Cooperia spp.	27	6	0	0
Haemonchus contortus	341	387	0	0
Nematodirus spp.	1	1	0	0
Oesophagostomum spp.	13	1	0	0
Ostertagia spp.	10	15	0	0
Trichostrongylus spp.	26	22	0	0

[1] Infection with the tapeworm *Moniezia expansa* was also found in lamb 309.
[2] Placed on part of pasture 2 weeks after infected ewes were removed.
[3] Placed on a second part of pasture about 2 months after infected ewes were removed.
[4] Placed on a third part of the pasture 4 months after infected ewes were removed.

On August 19, lamb 309, 5 months old, was placed on a second one-sixth part of this pasture. Fecal examinations after the 2 weeks of grazing demonstrated no nematode eggs. Lamb 322, 8 months old, was allowed to graze from October 16 to October 30 on a third part of this pasture. No eggs were found on subsequent fecal examinations, and no larvae developed in cultures prepared from the feces of this animal.

In the development and survival of nematode larvae, the results of experiment 3 support those of the two previous experiments, even though in the three experiments the pastures varied with respect to the amount of grass and shade. None of these experiments show the exact time necessary for the disappearance of infective nematode larvae. Experiment 1 shows that the time necessary for the pasture to become free from the preparasitic stages of the nematodes originally present was less than 3½ months. Experiment 2 reduces this period to less than 2½ months, and experiment 3 shows that the time may be only 2 months, and that even the presence of an abundance of shade and a heavy growth of grass did not protect the early stages of the nematodes in question.

SURVIVAL OF NEMATODES ON PASTURE DURING LATE FALL AND WINTER (EXPERIMENT 4)

When it was found, on November 14, 1938, that the pasture used in experiment 1 was apparently free of infective stages of all sheep nematodes except *Trichuris ovis*, eight heavily infested sheep were put on it. These sheep were naturally infected with *Bunostomum*, *Chabertia*, *Cooperia*, *Haemonchus*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, and *Trichostrongylus*. The animals were left on pasture from

November 14 to November 28. On November 29, two 7-month-old lambs (Nos. 193 and 200), together with four sheep heavily infested with the common ovine nematodes, were placed on the entire pasture. The four sheep were placed on the pasture at the same time as the lambs to increase the chance for transmission of parasites. The four sheep were removed after 10 days and the lambs after 14 days. The latter were kept for 2 weeks in a concrete-floored pen. No eggs appeared in the feces and no larvae were recovered from the cultures. The lambs were then returned to the same pasture and kept there for an additional period of 2 weeks. Both fecal examinations and fecal cultures showed the lambs to be negative for nematode eggs and larvae.

On March 27, 1939, two 12-month-old lambs (Nos. 161 and 199) were placed on this pasture and allowed to graze for 2 weeks. As no infection developed, they were returned to the pasture on May 23 and removed on June 6. The cultures of feces of the lambs then showed a very light infection, each lamb having three *Ostertagia* larvae and lamb 199 having 6 *Trichostrongylus* larvae. No eggs were found when the feces were examined. This experiment indicated that no stages of the common sheep nematodes developed to infectivity during the winter that this study was made. However, a small number of some of the preinfective stages survived the winter and developed to the infective stage in the spring.

SURVIVAL OF NEMATODES ON PASTURE IN THE EARLY SPRING (EXPERIMENT 5)

Five ewes heavily infested with *Bunostomum*, *Chabertia*, *Cooperia*, *Haemonchus*, *Müllerius*, *Nematodirus*, *Oesophagostomum*, *Ostertagia*, *Strongyloides*, and *Trichostrongylus* were placed, on March 8, 1939, on the pasture used in the third experiment. The sheep were removed on March 27. Two 12-month-old lambs (Nos. 190 and 196) were put on the entire pasture on March 27 and removed on April 10. No infection developed. They were returned to this pasture on May 23 and left there until June 6. Lamb 190 remained uninfected, but the feces of lamb 196 contained a few eggs and two larvae of *Nematodirus* spp., and four larvae of *Ostertagia* spp. developed in cultures made on June 13. This experiment showed that in the region where this work was done, the spring of 1939 was not only too cold for the development of the larvae of the other genera named but that even their preparasitic stages were killed.

DISCUSSION

The experiments reported in this paper support the views of Leiper and Taylor that larvae of nematodes parasitic in sheep die rapidly in the open, and they demonstrate that the danger of infection from these larvae rapidly diminishes in unused pastures during the summer and late spring. The question of survival of larvae during the winter was not settled. However, the fact that the larvae died rapidly in the spring and fall indicated that even if they did survive the winter, they would die soon afterwards.

Baker's experiments (*1*) are scarcely comparable with those reported in this paper. In his studies, uninfected lambs were placed on pasture infected 12 months previously and left on the test pasture for 3 months. Since the life cycle of most of the forms of nematodes found in his

experiments requires about 3 weeks for completion, there would have been ample time for several generations of worms to develop while the sheep were on pasture. Hence Baker's experiments give no data on the actual number of larvae surviving. An infection too light to be found by standard fecal diagnostic methods would have time to develop to a relatively heavy proportion in 3 months. The infective stages of nematodes, namely, *Nematodirus*, *Ostertagia*, and *Trichostrongylus*, that Griffiths found surviving from October till May, are the ones that survived cold weather in the present series of experiments.

No information on the longevity of the eggs of *Trichuris ovis* was found in the literature, but this form survived for the duration of the tests reported in this paper.

SUMMARY AND CONCLUSIONS

To obtain data on the period of survival of the common nematodes of sheep, experiments were conducted at different periods of the year and on pastures containing various amounts of grass and shade. Lambs uninfected with nematodes except *Strongyloides* were used as test animals. They were placed on the pastures at various intervals after the removal of heavily infested ewes. The experiments were carried on in 1938 and 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

In the experiments on summer pastures, the first showed that the time necessary for the pastures to become free from preparasitic stages of the nematodes originally present was less than 3½ months. The second experiment reduced this period to less than 2½ months. The third experiment showed that the time may be only 2 months, and that even the presence of an abundance of shade and a heavy growth of grass may not protect the early stages of the nematodes.

In the winter or late fall, there was no development to infectivity of preparasitic stages of ovine nematodes, but a small number of *Ostertagia* and *Trichostrongylus* in the egg or preinfective larval stage survived and developed to the infective stage the following spring.

There was evidence that preinfective stages of *Nematodirus* and *Ostertagia* survived the cold weather of early spring but did not become infective until late in the spring.

Eggs of *Trichuris ovis* survived on the pasture for as long as 3½ months in summer.

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COLLAR ROT RESISTANCE IN TOMATOES[1]

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INTRODUCTION

The disease of tomato foliage caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout and known as early blight occurs practically everywhere in the United States of America and in most other countries where tomatoes are grown. A tomato crop is seldom free of early blight, and losses caused by it are sometimes extremely high. Thus it is familiar to all commercial tomato growers. Since all varieties of tomatoes have appeared to be susceptible to early blight, the prospect of breeding resistant varieties has not appeared very hopeful. Control of the disease has usually been approached through changes in crop management and the application of fungicidal dusts and sprays.

The collar rot phase of *Alternaria solani* infection is generally less familiar to tomato growers. It consists of dark sunken lesions or cankers girdling the stems of young plants at or near the soil level.[2] It develops on plants in the seedbeds or coldframes, during shipment and after transplantation to the field, and on plants seeded directly in the field.[3] [4] Many plants are killed, and many others break over at the weakened area of the stem, remain flat on the soil, and put out new roots above the collar lesion. These are able to survive but fail to develop into vigorous plants.

The collar rot phase of *Alternaria solani* infection of tomatoes has become increasingly important along with the practice of shipping tomato plants from Southern to Northern States. Only a comparatively few commercial varieties, such as Marglobe, Rutgers, Indiana Baltimore, John Baer, and Grothen Red Globe, are involved in this plant-shipping industry. Studies at the United States Regional Vegetable Breeding Laboratory, Charleston, S. C., show that these varieties so far as examined are highly susceptible to collar rot, whereas certain other varieties are outstandingly resistant. It appears, therefore, that the control of collar rot may be effectively approached through plant breeding.

The purpose of this paper is to record the collar rot reaction of a large number of tomato varieties and selections and to describe the methods by which it has been possible to determine varietal differences in resistance.

[1] Received for publication January 30, 1942. This work was performed at the U. S. Regional Vegetable Breeding Laboratory, Charleston, S. C., chiefly under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935.

[2] PRITCHARD, F. J., and PORTE, W. S. COLLAR-ROT OF TOMATO. Jour. Agr. Res. 21: 179–184, illus. 1921.

[3] THOMAS, H. R. COLLAR-ROT INFECTION ON DIRECT-SEEDED TOMATOES. U. S. Bur. Plant Indus., Plant Dis. Rptr. 24 (1): 8–10. 1940. [Processed.]

[4] THOMAS, H. R., and SAMSON, R. W. DISEASES OF CANNING TOMATOES IN INDIANA AND THE RELATION TO SOURCE OF TRANSPLANTS. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22. 330–331. 1938. [Processed.]

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill.) plants were produced in flats in which they were allowed to grow for 4 weeks. During that time they were thinned to permit normal uniform growth. At the end of 4 weeks they were taken up, inoculated, and transplanted to greenhouse bench soil consisting of unused or sterilized compost. The fresh compost was fairly light, well-screened, and practically free of damping-off organisms, so that no complications with other organisms were encountered.

Inoculation consisted in dipping the tops, including the stems, of the seedlings in a water suspension of a macerated culture of *Alternaria solani*. The culture employed was the same strain as that used in early blight studies made prior to these on collar rot.[5] Roots were not immersed. Ten plants were dipped in the inoculum at a time. Plants were set immediately to a depth of about 3 inches in rows in the bench soil, furrows were made between rows, and water was applied in the furrows in such a way as not to wash any inoculum from the stems. Collar rot readings were made on the 7th and 14th days. Usually 100 percent collar rot infection developed on susceptible varieties within 7 days. Since the inoculated plants were not held in damp chambers but were kept on open benches at all times, the foliage infection was kept at a minimum and in fact developed scarcely at all.

The alternaria culture was grown in liter flasks of liquid medium[6] and was used when 3 to 4 weeks old. The entire culture was thoroughly macerated or broken into fine particles with the assistance of an electric homogenizer or blending apparatus.[7] The macerated culture was diluted to about four volumes with distilled water. Enough inoculum was prepared so that a fresh quantity could be used for each series of plants inoculated.

Final collar rot evaluations were made by (1) recording the number of survivors, (2) pulling the survivors, washing the stems free of soil, and recording the degree of collar infection, and (3) calculating the relative tolerance to collar rot. The index of tolerance was based on the following assignment of values: 0, plants killed; 25, plants alive but broken over at collar lesion; 50, plants with well-developed collar lesions but still erect; 75, collar lesions very shallow or with a definite tendency to heal; and 100, no collar lesions. Final rating of varieties thus took into account not only survival and freedom from collar rot but also the extent of development or severity of collar rot lesions and the apparent ability of some varieties to recover or heal after infection.

The high effectiveness of the technique was proved not only by visual observation but also by the high F value obtained in variance analysis (see table 1).

RESULTS OF COLLAR ROT INOCULATIONS

The varieties, accessions, and clonal selections listed in tables 1 and 2 are arranged in descending order of resistance to collar rot. Table

[5] ANDRUS, C. F., REYNARD, G. B., and WADE, B. L. RELATIVE RESISTANCE OF TOMATO VARIETIES, SELECTIONS, AND CROSSES TO DEFOLIATION BY ALTERNARIA AND STEMPHYLIUM. [U. S. Dept. Agr. Cir. 652.]

[6] The liquid medium consisted of 10 gm. of peptone, 0.5 gm. of monopotassium phosphate, 0.25 gm. of magnesium sulfate, 20 gm. of sucrose, and 1,000 cc. of distilled water.

[7] ANDRUS, C. F. PREPARATION OF INOCULUM WITH A MECHANICAL LIQUEFIER. Phytopathology (Phytopath. Note) 31: 566-567. 1941.

1 shows varietal ranks following variance analysis of one complete four-replicate experiment. Table 2 includes additional varieties tested for collar rot reaction but not in the replicated experiment. Some of the varieties listed as resistant or tolerant showed considerably less than 100 percent freedom from collar rot. This is because a few plants of those varieties actually bore collar rot lesions, but the lesions were superficial and contributed little to the disability of the plant. Several grades of collar rot injury following inoculation are shown in figures 1 and 2.

Most of the varieties listed as resistant or tolerant in table 1 are relatively unknown to tomato growers in this country. They are predominantly from continental Europe or England. Those of the greatest probable value for immediate adaptability are the English forcing varieties Ailsa Craig, Devon Surprize, and King George, and the Danish varieties Danish Extra Early and Danish Export—all medium-sized red tomatoes that have proved to be very productive in South Carolina. Other varieties listed as resistant or tolerant to collar rot in table 1 include Norduke, of high quality and reputedly resistant to fusarium wilt, at least in certain areas, and Riverside, one of the newer wilt-resistant varieties, which is useful principally as a canning tomato (figs. 1 and 2).

TABLE 1.—*Collar rot rating of 115 tomato varieties and selections based on a four-replicate inoculation with Alternaria solani*

Group and designation	Vegetable Breeding Laboratory accession No.	Source [1]	Rating [2]
Group 1 (resistant or tolerant):			
Semperfructifera	99	Europe	98
P. I.[3] 118787	812-M1	South America	96
P. I. 117228	795-M1	Asia	95
Erste Ernte	54	Europe	94
King George selection	207-M1	England	94
P. I. 119105	817-M1	South America	94
Targinnie Red Selection	6-02-M4	Australia	92
Laxtons Open Air [4]	82	England	92
Tomato No. 73	780	North America	92
P. I. 127467	1005-M1	Asia	92
King George	207	England	91
Danish Extra Early selection	30-M2	Europe	90
Devon Surprize	35	England	90
Soleil Levant	97	Europe	90
Ailsa Craig	211	England	90
Cherry	781	North America	90
P. I. 120273	836-M1	Asia	89
Dobbies Champion [4]	45	Scotland	88
Lycopersicon pimpinellifolium × Marglobe (F_1).	245-2-9-1-M1	U. S. Regional Vegetable Breeding Laboratory.	88
Danish Extra Early	30	Europe	87
Targinnie Red	6-7	Australia	86
Danish Export	31	Europe	85
P. I. 126913	384-M5	South America	85
P. I. 118789	814-M1	do	85
Targinnie Red selection	6-02-M5	Australia	82
Do	6-10-M1	do	82
P. I. 138628	1027-M1	Asia	80
Norduke	202	North America	78
Riverside	973	do	78
Cereza	34	South America	77
P. I. 134208	882-M2	Asia	77
Lucullus	80	Europe	75
P. I. 120260	829-M1	Asia	74
Essex Wonder	52	England	73
Danish Extra Early selection	30-M1	Europe	70
Lister Excelsior	2	North America	68
Prairiana	214	do	64

[1] The source mentioned represents the best information available to the authors. The actual locality of origin of many of the named varieties is obscure.

[2] Significant difference mean of 4=16.03 at 5 percent; 21.07 at 1 percent. F_s=41.41**.

[3] P. I. refers to accession numbers of the Division of Plant Exploration and Introduction.

[4] Probably not true to type.

TABLE 1.—*Collar rot rating of 115 tomato varieties and selections based on a four replicate inoculation with Alternaria solani*—Continued

Group and designation	Vegetable Breeding Laboratory accession No.	Source	Rating
Group 2 (susceptible or segregating):			
Targinnie Red selection	6-02-M8	Australia	59
Lycopersicon pimpinellifolium × Marglobe (F_4).	244-5-3-M3	U. S. Regional Vegetable Breeding Laboratory.	59
Lycopersicon pimpinellifolium	510-M3	South America	48
P. I. 79532 selection	7-010-2-M1	do	47
Lycopersicon peruvianum	543	do	45
Do	549	do	43
P. I. 79532 selection	8-M1	do	41
Victor Emanuel	108	Europe	41
Targinnie Red selection	6-02-S3	Australia	37
Lycopersicon pimpinellifolium	590-M3	South America	37
P. I. 79532 selection	7-013-5-M4	do	36
Gilbertiana	70-7	North America	33
P. I. 79532 selection	7-04	South America	32
Lycopersicon pimpinellifolium × Marglobe (F_4).	244-18-3-M1	U. S. Regional Vegetable Breeding Laboratory.	32
Lycopersicon peruvianum	537	South America	30
Lycopersicon pimpinellifolium × Marglobe (F_3).	575-M5	U. S. Regional Vegetable Breeding Laboratory.	30
P. I. 79532 selection	8-06-1	South America	23
Lycopersicon pimpinellifolium × Marglobe (F_5).	245-2-9-1-M3	U. S. Regional Vegetable Breeding Laboratory.	19
Bay State	571	North America	19
Group 3 (very susceptible):			
P. I. 79532 selection	8-13-M1	South America	15
Nystate	1035	North America	14
Lycopersicon pimpinellifolium × Marglobe (F_5).	244-2-7-3-M1	U. S. Regional Vegetable Breeding Laboratory.	13
Marglobe × *Lycopersicon pimpinellifolium* (F_5).	246-0-1-6-M1	do	13
Columbia	209	North America	12
Mingold	1040	do	11
Gulf State Market	222	do	9
Bounty	1046	do	8
Marglobe selection	9-01	do	5
Commercial Marglobe selection	10-02	do	5
Adelaide Dwarf	13	Australia	5
Express	50	do	5
Early Baltimore	201	North America	5
Marvel	217	do	5
Stone	235	do	5
Grothen Red Globe	238	do	5
Early Baltimore	1041	do	5
Riverside Favorite	85	Europe	4
Indiana Certified Marglobe	195	North America	4
Rutgers	200	do	4
Stokesdale	242a	do	4
Marglobe × Fiaschello (F_4)	254-0-2-M1	U. S. Regional Vegetable Breeding Laboratory.	4
John Baer	888	North America	4
Vetomold	970	do	4
Early Shipper	1038	do	4
Master Marglobe	228	do	3
Louisiana Gulf State	231	do	3
Fiaschello × Marglobe (F_4)	255-5-3-M2	U. S. Regional Vegetable Breeding Laboratory.	3
Essar	890	North America	3
Globelle	975	do	
Glovel	1031	do	3
Bred-Rite Marglobe	1033	do	3
Kilgores New "X"	1034	do	3
Fiaschello	56	Europe	2
San Marzano	128a	do	2
Pritchard	192	North America	2
Glovel	216	do	2
Marglobe × Fiaschello (F_3)	254-1-6-3-M1	U. S. Regional Vegetable Breeding Laboratory.	2
Do	254-5-3-M1	do	2
US23W(b20) selection	624-M1	North America	2
Do	624-M2	do	2
Do	624-M4	do	2
Pan America	889	do	2
Marhio	974	do	2
Valiant	241	do	1
Marglobe × *Lycopersicon pimpinellifolium* (F_5).	246-0-1-6-M3	U. S. Regional Vegetable Breeding Laboratory.	1
Fiaschello × Marglobe (F_5)	255-1-1-1-M1	do	1
Victor	971	North America	1
Essary	980	do	1

TABLE 1.—*Collar rot rating of 115 tomato varieties and selections based on a four-replicate inoculation with Alternaria solani*—Continued

Group and designation	Vegetable Breeding Laboratory accession No.	Source	Rating
Group 3 (very susceptible)—Continued.		North America	
Ponderosa	1029	do	1
Cuban Marglobe	1030	do	1
Crown selected Marglobe	1032	do	1
Break o' Day	197	do	0
Marvelosa	206	do	0
Cooper Special	223	do	0
Montgomery	234	do	0
Earliana	239a	do	0
McGee	1039	do	0
Early Santa Clara	1043	do	0

TABLE 2.—*Collar rot ratings of 42 tomato varieties and crosses not included in the analysis of variance*

Group and designation	Vegetable Breeding Laboratory accession No.	Source [1]	Rating
Group 1 (resistant or tolerant):			
P. I. 95558 × Targinnie Red	913–M1	U. S. Regional Vegetable Breeding Laboratory.	80
Group 2 (susceptible or segregating):			
Stoners M. P	95	Scotland	52
El Colono	37	South America	46
Heterosis	64	Europe	40
Trivetts Abundance	93	North America	36
Fillbasket	224	England	32
Bonny Best	243	North America	30
Group 3 (very susceptible):			
Everbearing	570	do	23
Chinaman × Marglobe (F_1)	225	do	22
Magnum Bonum	78	Europe	22
Browns Special	1042	North America	21
Red Cap	1036	do	21
Il Duce [2]	59	Europe	20
Everbearing Scarlet Globe	220	North America	20
Danmark	29	Europe	19
Sterling Castle	205	England	19
Marglobe × Chinaman (F_1)	226	U. S. Regional Vegetable Breeding Laboratory.	17
Chemin Early Red	16	Europe	16
Amarillo	68	South America	16
Kondine Red	63	England	15
Red Rock	236	North America	14
Original Pomona	118	Europe	13
Indefatigable	26	do	13
Up-to-Date	88	England	13
Rouge Grosse Native	127	Europe	12
Nicholsons Dallas	210	North America	12
Pearson	1044	do	11
Amwell	11	Europe	11
Earliest of All	38	North America	11
Radio	114	do	11
In Miscuglio	58	Europe	10
Pierrette	122	do	10
Louisiana Dixie	230	North America	9
Novato	72	Europe	9
Cameron Canada (Nova Scotia)	24	North America	8
Chinaman	227	do	8
Augusta	23	Europe	6
Burwood Prize	21	North America	5
Tuckswood	91	Europe	5
Marshalls Prolific	75	do	4
Market Wonder	76	do	4
Resista	111	do	4

[1] The actual locality of origin of many of the named varieties is obscure.
[2] Probably not true to type.

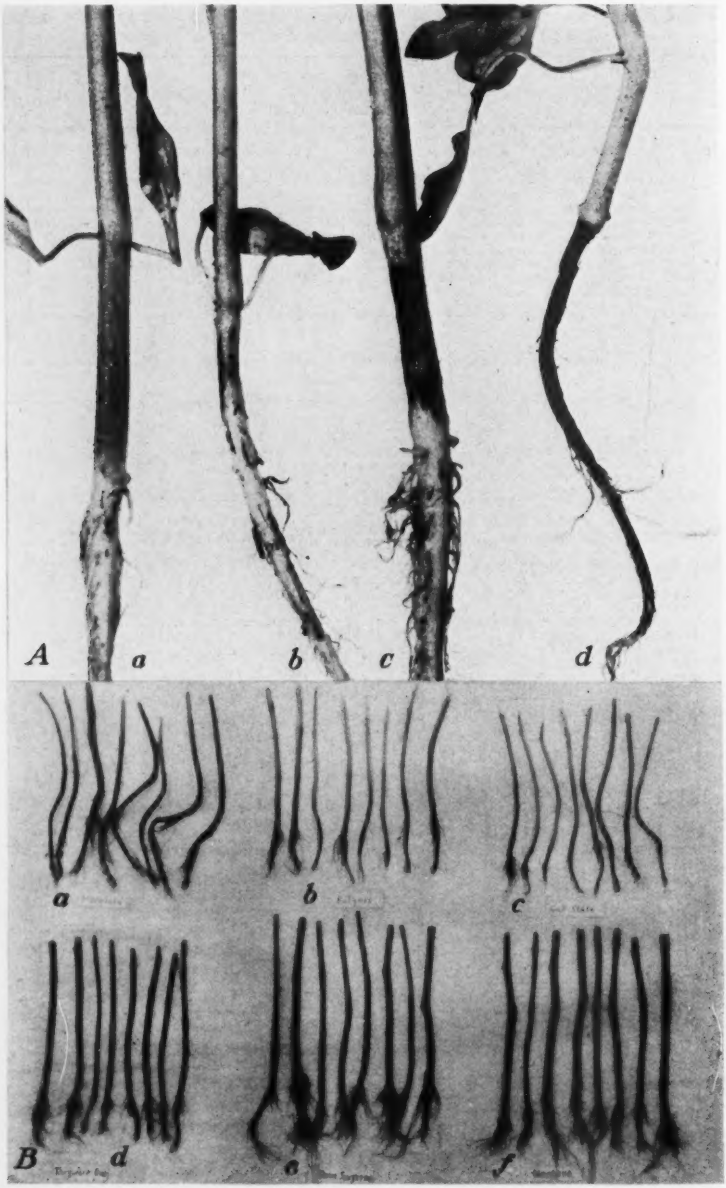

FIGURE 1.—*A*, Basal parts of inoculated tomato plants illustrating various collar rot ratings: *a*, 100 (no collar lesions); *b*, 75; *c*, 50; and *d*, 25. *B*, Reaction of three susceptible varieties: *a*, Marglobe; *b*, Rutgers; and *c*, Gulf State Market; and of three resistant or tolerant varieties: *d*, Targinnie Red; *e*, Devon Surprize; and *f*, Norduke. *A*, × 1; *B*, × ⅛.

Definite evidence of segregation for collar rot resistance is shown in the varieties Columbia, Prairiana, Gulf State Market, and Early Baltimore. The fact that different clonal selections by cuttings of

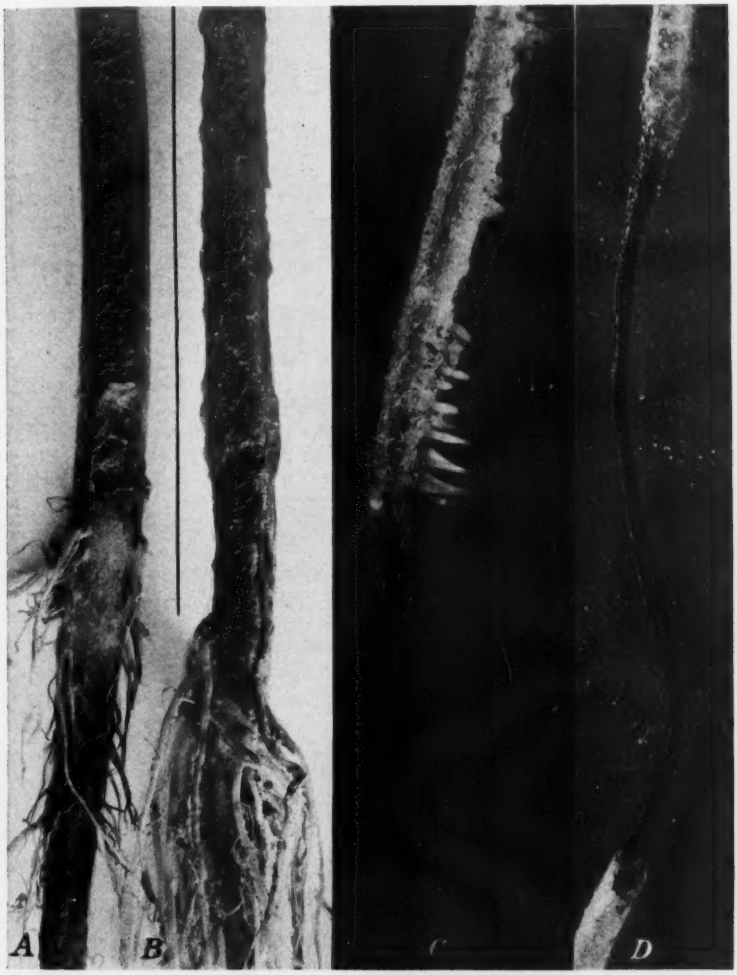

FIGURE 2.—Collar lesions of various types caused by *Alternaria solani: A*, Tolerant-reaction type; *B*, well-developed lesion showing a tendency to heal; *C*, severely infected collar with new roots developing above the lesion; *D*, severely infected collar without root development. All, × 3.

several varieties, such as Targinnie Red and Danish Extra Early, fall into separate reaction classes points to the desirability of making rigid selections for collar rot resistance in the segregating varieties. From the evidence available it seems probable that the varieties

Norduke, Prairiana, and Riverside, for example, could all be raised to the level of highly resistant varieties by clonal selection.

Several lines of *Lycopersicon pimpinellifolium* are listed among those segregating for collar rot resistance. The presence of factors for resistance to collar rot in *L. pimpinellifolium* is proved further by tomato No. 245–2–9–1–M1$_1$ which is a fifth-generation selection from an *L. pimpinellifolium*×Marglobe cross. Tomato No. 575–M5 is a sample from an F_2 population of a similar cross.

DISCUSSION

Although earlier studies have shown that all varieties are susceptible to leaf infection by *Alternaria solani*, significant degrees of lesser susceptibility were found.[8] Many of the varieties listed herein as resistant or tolerant to collar rot were among those found to be significantly less susceptible to alternaria leaf spot. It is believed that the method devised for evaluating resistance to collar rot is of definite value in the larger problem of developing resistance to *Alternaria solani*.

In application of the foregoing information to a solution of the current collar rot problem several interesting possibilities are suggested:

(1) Substitution of the collar rot resistant or tolerant varieties Riverside, Norduke, or Prairiana for the highly susceptible varieties now preferred might be practicable under certain conditions.

(2) The considerable evidence that many desirable varieties are heterogeneous and segregating for collar rot resistance suggests that direct selection might quickly yield resistant strains of the desirable type.

(3) A breeding program involving judicious crosses between collar rot resistant and the more desirable but susceptible varieties appears entirely practicable. Hybrids of Devon Surprize × Marglobe, Devon Surprize × Montgomery, and Targinnie Red×Montgomery are now available in the second generation.

SUMMARY

A method of dipping foliage of 4-week-old tomato seedlings in a water suspension of macerated *Alternaria solani* culture at the time of transplanting is described. The method produced 100 percent collar rot on the most susceptible varieties and 100 percent freedom from collar rot on the most resistant.

A large number of standard tomato varieties and clonal selections are rated according to collar rot reaction. Apparently complete resistance to collar rot was found in 23 varieties and accessions.

Collar rot resistance is correlated with lesser degrees of susceptibility to alternaria leaf spot, and the method of determining collar rot resistance should facilitate the breeding for resistance to defoliation in tomatoes.

[8] See footnote 5.

ALKALOIDS IN CERTAIN SPECIES AND INTERSPECIFIC HYBRIDS OF NICOTIANA[1]

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INTRODUCTION

Although it has been known for many years that the main alkaloid in the cultivated species *Nicotiana tabacum* L. and *N. rustica* L. is nicotine ($C_{10}H_{14}N_2$), comparatively little analytical chemical work has been done on the wild species of *Nicotiana.* In 1934 Shmuk (*15*)[2] stated that *N. glauca* contained an alkaloid other than nicotine that was not identified. The following year Smith (*18*) identified the principal alkaloid in this species as anabasine, beta-pyridyl-α'-piperidine. Concurrently, Shmuk and Khmura (*17*) reported that certain other species of *Nicotiana* contained alkaloids whose picrates had melting points different from that of nicotine, but again they were not identified. *N. sylvestris* and crosses of this species with *N. tabacum* were discussed in some detail.

In 1937 Smith (*19*) determined the main alkaloid in *Nicotiana sylvestris* to be nornicotine ($C_9H_{12}N_2$), and at approximately the same time Shmuk (*16*) reported:

> It is known that alcaloids differing in chemical structure are contained in different species of *Nicotiana;* thus, *N. tabacum, N. rustica, N. alata, N. Langsdorffii* all contain nicotine; *N. sylvestris* and *N. Rusbyi* [*N. tomentosiformis* Goodsp.] contain an alcaloid belonging to the secondary bases—probably nornicotine, while *N. glauca* contains anabasine.

Nicotine has been found also in *Nicotiana attenuata* (*1*), *N. suaveolens* (*13*), and *N. trigonophylla* (*10*).

The investigations cited above constitute most of the relevant work that had been published on alkaloids in wild species of *Nicotiana,* not including hybrids, at the time this paper was written. The experiments hereafter described were begun in 1936. The object of the work was to determine the alkaloids in a large number of representative *Nicotiana* species and hybrids among different alkaloidal types; to perfect chemical methods for isolating and identifying alkaloids found in *Nicotiana;* and to consider the results obtained from the standpoint of developing new strains yielding larger amounts of alkaloids for possible use in insecticides.

Minute quantities of 11 or more secondary alkaloids, in addition to the principal one, nicotine, have been reported in the cultivated species *Nicotiana tabacum* (*4*); but of these only 8 have been confirmed by Späth and Kesztler (*23*). It has also been found that although most of the total alkaloidal content in *N. sylvestris* is nornicotine, there is a small percentage of *l*-nicotine (*16, 19*). The investigations reported here indicate that in many, if not all, species of *Nicotiana* the principal alkaloid is accompanied by 1 or more secondary forms, usually present in very small amounts.

[1] Received for publication February 12, 1942.
[2] Italic numbers in parentheses refer to Literature Cited, p. 358.

The three predominant alkaloids in *Nicotiana*, namely anabasine, nornicotine, and nicotine, are not peculiar to this genus. Anabasine was discovered in *Anabasis aphylla* L., of the Chenopodiaceae (*12*); nornicotine has been found in *Duboisia hopwoodii* F. Muell., of the Solanaceae (*5*); and nicotine has been reported to occur in *Asclepias syriaca* L., of the Asclepiadaceae (*8*), although in the last-named species only a minute quantity of the alkaloid was indicated.

CULTURAL METHODS

All plants in these experiments were grown at the Arlington Experiment Farm, Arlington, Va.[3] The seedlings were planted in the field, 18 inches apart in rows 1 meter apart. Shortly before planting time, 4–8–4 fertilizer was applied uniformly in the drill at the rate of 800 pounds to the acre. The soil was Keyport silt loam, a heavy claylike type.

Many of the species were not topped and suckered as is usual in the culture of leaf tobacco, since their habit of growth was not adapted to this method of handling. Differences in cultural treatment are known to affect alkaloidal content, so that the total percentages reported for the 29 species are not considered to be strictly comparable. The identity of the alkaloids and their approximate relative amounts may be considered as characteristic.

All samples were air-dried and ground into a fine powder preparatory to chemical analysis. The samples comprised both leaves and stems in all species except the larger ones; namely, *Nicotiana benavidesii*, *N. glauca*, *N. otophora*, *N. raimondii*, *N. sylvestris*, *N. tomentosa*, *N. tomentosiformis*, and *N. wigandioides*, in which only the leaves were analyzed.

CHEMICAL METHODS

The determination and separation of the alkaloids were effected by the following procedures.

A 50-gm. sample was digested with water in a liter Erlenmeyer flask at the highest temperature of the steam bath for about 2 hours. The unextracted material was filtered from the solution and washed with several portions of boiling water. The extractions were combined, evaporated to a low volume on the steam bath, made strongly alkaline with sodium hydroxide, and then extracted with several portions of ether. The ether extractions were combined, and the alkaloid was washed out with dilute hydrochloric acid. The washed ether was used repeatedly to extract more alkaloidal material from the alkaline extract. Repeated extractions were made because persistent and incompletely separated emulsions usually formed. Their formation was reduced to a minimum by making the extractions in Erlenmeyer flasks, decanting after each extraction, and avoiding violent shaking. Careful manipulation usually permitted complete extraction and the final clearing of the emulsion.

The acid solution of the alkaloids was separated from the ether, made strongly alkaline, and extracted with several small portions of

[3] Seeds of many of the species grown were made available through the kindness of Dr. T. H. Goodspeed, of the University of California. Some were obtained from Dr. R. E. Clausen, of the same institution. The remainder formed part of the collection maintained by the Division of Tobacco Investigations.

ether. The ether extractions were combined and dried for several hours or overnight with sticks of sodium hydroxide or preferably potassium hydroxide pellets. The dried ethereal solution was evaporated in a 50-cc. tared Erlenmeyer flask and subjected to the direct steam of the bath until all traces of alcohol and ether had been removed, the period of heating being no longer than necessary. The flask with residue was dried over sulfuric acid in a desiccator for 18 hours and was then weighed.

In the procedure just outlined, complete extraction by hot water may not be effected, but approximately 95 percent of the alkaloidal content is removed. Variations, such as the addition of oxalic acid or calcium carbonate (10 gm. to 700 cc. of water) followed by precipitation of the soluble lime by acidification with oxalic acid, did not appreciably affect the values found for total alkaloids.

In separating and identifying the alkaloids, nicotine was removed first by distillation through an efficient fractionating column such as the Widmer. The method is based on the discovery by one of the writers (*20*) that nicotine forms an azeotropic mixture with water at the boiling temperature when 2.5 gm. are contained in 100 cc. Neither anabasine nor nornicotine forms a similar mixture, nor does any other alkaloid so far encountered in the genus *Nicotiana*. The procedure is as follows.

The total alkaloids were washed into the distilling flask with 75 to 100 cc. of water and distilled slowly through the column until only about one-fifth or less of the volume remained. Sixty to eighty cubic centimeters of water was added to the mixture, which was then distilled again. The combined distillate represented all the nicotine together with some "nonvolatile" alkaloid and was titrated with standard acid to obtain the approximate amount of nicotine. The titrated distillate was made alkaline with standard alkali in slight excess of that required by the acid titration, and the distillations were repeated. The titration of this distillate gave a close check on the amount of nicotine contained and was somewhat less than the first titration if some nonvolatile alkaloid had distilled over with the nicotine. This highly purified second nicotine distillate was made appreciably acid to methyl red, evaporated to a low volume if necessary, and picric acid solution was added to form nicotine picrate.

In all species containing 0.2 percent or more of total alkaloids, melting-point determinations were made with mixtures of pure nicotine picrate. It is safe to conclude from the many melting points made that any alkaloid distilled under the conditions outlined will give the characteristic needles of nicotine picrate and that, when mixed with a known amount of nicotine picrate, the melting point will not be changed. Any residual nonvolatile alkaloid was precipitated as the picrate, which was recrystallized at least once. The picrate was recrystallized from water several times if sufficient material was available, and crops of crystals were formed of which the melting points were obtained. The presence of anabasine or nornicotine was indicated by the crystalline form and melting point. In no single species did both of these alkaloids occur together. With certain hybrids, however, both have been found along with nicotine, and careful fractionation was necessary to confirm their identification. Actual results with these hybrids are given below to show the basis

for the conclusions. All melting points given are corrected for stem exposure.

In the F_1 hybrid *Nicotiana tabacum* × *N. glauca* (38114) we might expect only a mixture of anabasine and nicotine, but careful fractionation of the picrate of the nonvolatile alkaloids into (1) most soluble, (2) less soluble, and (3) least soluble yielded fractions with melting points of 188°, 194°, and 205° to 206° C. respectively. Recrystallized fraction 1 melted at 188° to 190° and was unchanged when mixed with nornicotine picrate. When fraction 3 was recrystallized it melted at 207° to 209°; this melting point remained unchanged when fraction 3 was mixed with racemic anabasine picrate. The specific rotation $[\alpha]_D$ of the nonvolatile alkaloid in acid solution was +7.8°. It is probable that this optical activity is due to the presence of *l*-nornicotine, which is dextrorotatory in its salts (*l*-nicotine and *l*-anabasine are also dextrorotatory in their salts). Anabasine in *N. glauca* has always been found to be essentially optically inactive.

The F_1 hybrid *Nicotiana tabacum* × *N. glauca* (39115–1) contained a little nicotine; the picrate of the nonvolatile alkaloid melted at 201° to 202° C., which was raised on recrystallization to 205° to 206°; and the melting point of a mixture with racemic anabasine picrate was 208° to 209°. Optical rotation of the nonvolatile alkaloids was $[\alpha]_D$ −20° to 20.8°. The conclusion was drawn that this hybrid contained essentially *d*, *l*-anabasine with an excess of *l*-anabasine, since no crystals resembling nornicotine picrate could be obtained. It is evident that a quantitative method for separating anabasine and nornicotine is needed before final conclusions can be drawn regarding the composition of the hybrids of *N. glauca* with *N. tabacum* or any plant containing nicotine and nornicotine.

All samples of *Nicotiana glauca* that the writers have examined contained principally optically inactive anabasine, the picrate of which melts at 213° C. The optical activity is occasionally very slightly levorotatory, which probably indicates that a small excess of *l*-anabasine is present.

The sample of *Nicotiana glauca* shown in table 1, as well as others not reported here, was found to contain some nicotine. No nicotine has been found, however, in samples of *N. glauca* obtained from the wild. It is possible, but doubtful, that the nicotine in the former may have been introduced from accidental absorption during the drying or pulverizing process. This point may be settled easily by careful isolation of the writers' cultivated material.

The nornicotine that occurs in several species, such as *Nicotiana sylvestris* and *N. glutinosa*, has been found to be the nearly pure levorotatory form. In a number of other species the partly racemized form probably occurs, the picrates of which have melting points that vary from 186° to 192° C. When the content of nornicotine was less than 0.2 percent its presence was indicated only by the appearance and properties of the picrate. The picrates, even after several recrystallizations, usually appear in the colloidal form, but on long standing form hard crystals which repeat this behavior on further recrystallization.

Observations on the optical rotation of the alkaloids as found in the writers' material are admittedly incomplete, but it seems that nicotine occurs always in the *l*- form, anabasine principally in the

optically inactive form, and nornicotine largely in the *l*- form mixed with varying proportions of the optically inactive form.

In the progress of this work the possible presence of new alkaloids has been kept in mind, but none has been indicated. The new alkaloid anatabin, discovered by Späth and Kesztler, is closely related to anabasine and was expected to occur in *Nicotiana glauca* or *N. glauca* hybrids, but so far no indication of its presence has been obtained. Since this study was necessarily confined to the principal alkaloids it is quite probable that very small proportions of other alkaloids were present but could have been detected only by using much more material for separation.

RESULTS

A total of 29 wild species of *Nicotiana* were studied of which 4 apparently contained only nicotine, 5 only nornicotine, 2 had mixtures of anabasine and nicotine, and the rest mixtures of nicotine and nornicotine (table 1). Anabasine was the main alkaloid and nicotine the secondary one in *N. debneyi* and *N. glauca*, the only species in which these 2 alkaloids were found together as the chief alkaloidal constituents.

TABLE 1.—*Alkaloids of some wild species of Nicotiana*

Species	Main alkaloid	Secondary alkaloid	Total alkaloidal content	Main alkaloid		Phylogenetic grouping
				Percent	Percentage of total alkaloid	
			Percent			
N. alata Lk. and Otto	Nicotine	None	0.06	0.06	100	*alata.*
N. benavidesii Goodsp	do	Nornicotine	.92	.82	89	*paniculata.*
N. bigelovii S. Wats	do	?	.18	.16	89	
N. cavanillesii Dun	Nornicotine	Nicotine [1]	.33	.33	100—	*cavanillesii.*
N. debneyi Domin	Anabasine	Nicotine	.46	.39	85	*suaveolens.*
N. exigua Wheeler	Nornicotine	do	.26	.24	92	Do.
N. glauca Grah	Anabasine	do	.64	.62	97	*paniculata.*
N. glutinosa L	Nornicotine	None	1.80	1.80	100	*tomentosa.*
N. goodspeedii Wheeler	do	Nicotine	.22	.21	95	*suaveolens.*
N. gossei Domin	Nicotine	None	1.10	1.10	100	Do.
N. langsdorffii Weinm	do	Nornicotine	.22	.16	73	*alata.*
N. longiflora Cav	do	do	.04	?	?	Do.
N. maritima Wheeler	Nornicotine	None	.28	.28	100	*suaveolens.*
N. megalosiphon H. and M	do	Nicotine [1]	.22	.22	100—	Do.
N. nesophila Johnst	do	Nicotine	.18	.17	94	*repanda.*
N. nudicaulis S. Wats	do	Nicotine [1]	.10	.10	100—	
N. otophora Griseb	do	?	.11	.11	100	*tomentosa.*
N. paniculata L	do	Nicotine	.28	.20	71	*paniculata.*
N. plumbaginifolia Viv	do	do	.02	?	?	*alata.*
N. raimondii Macbr	do	do	.80	.67	84	*paniculata.*
N. repanda Lehm	do	do	.40	.37	93	*repanda.*
N. stocktoni Brandeg	Nicotine	Nornicotine	.66	.46	70	Do.
N. suaveolens Lehm	Nornicotine	Nicotine	.46	.30	65	*suaveolens.*
N. sylvestris Speg. and Comes	do	Nicotine [1]	.44	.44	100—	
N. tomentosa Ruiz and Pav	do	None	Trace	Trace	100	*tomentosa.*
N. tomentosiformis Goodsp	do	Nicotine [1]	.08	.08	100—	Do.
N. trigonophylla Dun	do	None	.06	.06	100	
N. undulata Ruiz and Pav	do	Nicotine	.20	.17	85	*paniculata.*
N. wigandioides Koch and Fint	Nicotine	None	1.02	1.02	100	*tomentosa.*

[1] Only a trace of the secondary alkaloid was present.

Nicotiana longiflora and *N. plumbaginifolia* each contained both nicotine and nornicotine, but the amounts were so small that it was

not possible to determine which was present in the higher concentration. Among the 16 other species in which both nicotine and nornicotine were found, only *N. benavidesii*, *N. langsdorffii*, and *N. stocktoni* contained nicotine predominantly.

No wild species studied had as much as 2 percent alkaloidal content; whereas both the cultivated ones (*Nicotiana tabacum* and *N. rustica*) usually had at least this amount. Only 3 wild species gave analyses of over 1 percent alkaloid; and 22 had less than 0.5 percent alkaloid. In 23 species, 84 to 100 percent of the total alkaloidal content was taken up by the main alkaloid (table 1).

Hybrids were made between *Nicotiana* species in which different alkaloids were predominant so that crosses of nicotine types with nornicotine types, nicotine with anabasine, and anabasine with nornicotine could be investigated. Inheritance of alkaloids in F_1 hybrids of the first type was tested by crosses of *N. tabacum* (nicotine) with *N. sylvestris*, *N. tomentosa*, and *N. glutinosa* (all nornicotine species). The results are shown in table 2. In each hybrid the genetic factors controlling nornicotine formation were partly dominant over those producing nicotine. The total percentage of alkaloid in the F_1 plants was higher than in the nornicotine parents.

TABLE 2.—*Alkaloids of certain Nicotiana interspecific hybrids.*[1]

Nicotiana interspecific cross	Family No.	Main alkaloid	Secondary alkaloid	Total alkaloidal content	Main alkaloid	
					Percent	Percentage of total alkaloid
				Percent		
F_1 (*tabacum* × *sylvestris*)	3790	Nornicotine	Nicotine	2.26	2.25	99
Do	38104	do	do	1.14	.99	87
F_1 (*tabacum* × *tomentosa*)	39117	do	do	1.20	1.18	98
F_1 (*tabacum* × *glutinosa*)	38286	do	do	1.92	1.73	90
F_1 (*tabacum* × *glauca*)	39115	Anabasine	do	.96	.90	94
Amphidiploid (*tabacum* × *glauca*)	39116	do	do	.98	.95	97
F_1 (*glauca* × *tomentosa*)	38287	?	?	.24		
F_1 (*glutinosa* × *sylvestris*)	38113	Nornicotine	Nicotine	1.36	1.26	93

[1] All data in this table are on leaves of topped plants.

The F_1 hybrid *Nicotiana tabacum* × *N. glauca* was used to investigate a cross between a nicotine and an anabasine type. The genetic factors for anabasine formation are partly dominant over those controlling nicotine formation in the F_1 (table 2). The total percentage of alkaloid and of anabasine was higher in the hybrid than in *N. glauca*. In a sample of the *N. tabacum* × *N. glauca* hybrid grown in 1938 (38114) the writers have noted the presence of nornicotine in addition to anabasine and nicotine.

The F_1 *Nicotiana glauca* × *N. tomentosa* was intended to be used to investigate a cross between an anabasine and a nornicotine type. It was found, however, that with the present chemical techniques it was not possible to separate these two alkaloids readily in a mixed sample having such a small total alkaloidal content (0.24 percent).

The dominance of nornicotine over nicotine was considered to be only partial since neither *Nicotiana glutinosa* nor *N. tomentosa* contained detectable amounts of nicotine, yet this alkaloid was found in hybrids between each of these species and *N. tabacum*. Similarly the

genetic factors controlling anabasine formation probably are not completely dominant over those for nicotine since the percentage of anabasine in the total alkaloid is lower in the *N. tabacum* × *N. glauca* hybrid than in *N. glauca* itself.

Nicotiana glutinosa and *N. sylvestris*, two predominantly nornicotine species, were crossed, and, as expected, the alkaloidal content of the F_1 hybrid was mostly nornicotine with a small percentage of nicotine (table 2).

GENETIC DISCUSSION

SPECIES

Almost one-half of the definitely established species of the genus *Nicotiana* were included in these investigations. Seven of the fifteen recognized Australasian species were analyzed; and some from each of the four centers of distribution in the Western Hemisphere; namely, Peru and Bolivia, Chile and Argentina, Uruguay and southeastern Brazil, Mexico and southwestern United States. There was no apparent relation between the geographical distribution of a species and the identity of its main alkaloid.

Each of the known chromosome numbers (*2*), from those with 9 to those with 24 pairs of chromosomes, was represented by 1 or more species; and most of the main phylogenetic groupings in the genus (*3*) were included in the types used.

With regard to chromosome number, an interesting relation was observed in the Australasian or *suaveolens* group of *Nicotiana*. The principal alkaloid was found to be nornicotine in species that had 16 and 20 pairs of chromosomes; namely, *Nicotiana exigua*, *N. maritima*, *N. suaveolens*, *N. goodspeedii*, and *N. megalosiphon*. Anabasine was the main alkaloid in *N. debneyi*, the only Australasian species so far determined to have 24 pairs of chromosomes; and nicotine was the main alkaloid in *N. gossei*, the only species with 18 pairs of chromosomes.

Among American species, the cultivated *Nicotiana tabacum* and *N. rustica* have 24 pairs of chromosomes and contain a large amount of alkaloid, which is predominantly nicotine. *N. sylvestris*, *N. tomentosa*, and *N. tomentosiformis*, which are considered to be the probable wild progenitors of *N. tabacum* (*3*), have 12 pairs of chromosomes and contain a small amount of alkaloid, which is mainly or entirely nornicotine. Similarly, *N. paniculata* and *N. undulata*, which are considered to be the probable wild progenitors of *N. rustica*, have a small amount of alkaloid, which is predominantly nornicotine, and have 12 pairs of chromosomes.

The apparently inconsistent situation in which the hybridization of two species with low concentrations of alkaloid that is mainly nornicotine produces a new form having a high concentration of nicotine might be explained if evidence were available that the conditions under which the cultivated types were formed and are grown favor the production of large amounts of alkaloid and that high concentration of total alkaloid favors the formation of nicotine.

The following facts support the first part of this hypothesis: (1) There is experimental evidence that chromosome doubling (which is considered to have occurred in the formation of the cultivated species) causes an increase in alkaloidal content (*11*, *24*); (2) the cultural methods used in the growing of tobacco (topping, suckering, heavy

application of fertilizer) favor production of high alkaloidal content; (3) for centuries these species have been cultivated and used by man for the effect produced by the alkaloid that they contain. Therefore it seems not too speculative to assume that selection for high alkaloidal content (i. e., for genes that increase the production of alkaloid) has been practiced, and the fact that low-alkaloidal strains have been selected from some present-day varieties of *N. tabacum* supports this suggestion.

The following facts support the second part of the hypothesis; namely, that high concentrations of alkaloid favor the formation of nicotine (or low concentrations, the formation of nornicotine): (1) Large quantities of nornicotine have been found in low-alkaloidal strains of *Nicotiana tabacum* (*6*, *9*); (2) the alkaloidal content of species in which nicotine predominated (table 1) was higher on an average than those in which nornicotine was the main alkaloid; (3) Shmuk (*16*) found that, out of 11 F_1 plants of *N. tomentosiformis* × *N. sylvestris*, 9 had a total alkaloidal content ranging from 0.129 to 0.510 percent, while the other 2 had 0.656 and 0.859 percent, respectively, and that the chief alkaloid in the first 9 F_1 plants was nornicotine, while in the remaining 2 it was nicotine, thus showing that a predominantly nicotine type, like *N. tabacum*, could have been formed from the crossing of 2 predominantly nornicotine types, provided the hybrid had a sufficiently high concentration of total alkaloid.

In regard to relations among species in the various phylogenetic groups, it was found that of those analyzed in the *paniculata* group (*Nicotiana benavidesii*, *N. glauca*, *N. paniculata*, *N. raimondii*, and *N. undulata*), all but *N. glauca* were of predominantly nicotine or nornicotine types. From cytological observations (*3*), *N. glauca* had already been considered aberrant in this group. The discovery that it is exceptional for a species to have anabasine as the main alkaloid is confirmatory evidence of the isolated position of *N. glauca*. The fact that *N. debneyi* of the *suaveolens* group was the only other species found to contain anabasine as the principal alkaloid is evidently a case of parallel evolution rather than any indication of homology between this species and *N. glauca*. Most species of the *alata* group (*N. alata*, *N. langsdorffii*, *N. longiflora*, and *N. plumbaginifolia*) tend to contain nicotine predominantly; and most of the *tomentosa* group (*N. glutinosa*, *N. otophora*, *N. tomentosa*, *N. tomentosiformis*, and *N. wigandioides*), with one exception, nornicotine predominantly.

HYBRIDS

Russian investigators have reported in some detail on hybrids between different alkaloidal types, and with certain crosses the work has been continued to the F_2 and further inbred generations. Shmuk (*16*) has studied the following crosses: *Nicotiana tabacum* (nicotine) × *N. sylvestris* (nornicotine), *N. tomentosiformis* (nornicotine) × *N. sylvestris* (nornicotine), and *N. langsdorffii* (nicotine) × *N. alata* (nicotine). He also reported that anabasine dominated over nicotine in the F_1 hybrid *N. tabacum* × *N. glauca;* and that, in subsequent inbred generations, types with anabasine, nicotine, and mixtures of the two were produced.

A short time later Ternovsky, Khmura, and Žukov (*24*) published a paper on the alkaloids in various heteroploid types having extra chromosomal complements of *Nicotiana glauca* and of *N. tabacum*. All contained anabasine predominantly, even where there were two *N. tabacum* genomes to one of *N. glauca* ($24tt+12g$). This particular combination had a higher percentage of anabasine than others with proportionately more *N. glauca* chromosomes.

Ternovsky et al. (*24*) also investigated crosses between *Nicotiana rustica* and *N. glauca*. Only anabasine was found in the F_1, sesquidiploid ($24r+12gg$), and crosses of the sesquidiploid to *N. glauca* and *N. tabacum*. More recently Žukov (*25*) and Kostoff (*7*) have published results of their work on heteroploid *N. rustica* × *N. glauca* combinations. The amphidiploid ($24rr+12gg$) and the sesquidiploid ($24rr+12g$) had higher percentages of anabasine content than *N. glauca*. In backcrosses of the hybrid *N. rustica* × *N. glauca* to *N. rustica*, some plants were obtained containing only nicotine; some, only anabasine; and some, mixtures of nicotine and anabasine.

From their studies on the hybrids of *Nicotiana tabacum* with *N. sylvestris* and *N. glauca* the writers were able to confirm the general results reported by Shmuk and his coworkers, but their findings differ in certain respects from those of the latter. Shmuk (*16*) and Shmuk and Khmura (*17*) found no nicotine whatsoever in the cross of *N. tabacum* with *N. sylvestris*, whereas in all samples of this hybrid that the writers have analyzed some nicotine was present. It appears, however, that Shmuk was using the reciprocal cross, which conceivably might account for the different result. Shmuk and other Russian investigators found that in the F_1 hybrid *N. tabacum* × *N. glauca* nicotine was usually absent; but a few plants contained traces of this alkaloid. In analyses of this hybrid, the writers found that all samples contained some nicotine, and they consider it a characteristic secondary alkaloid of the F_1.

Results of the Russian investigations, which were continued beyond the first generation, may be summarized as follows: (1) Some segregants were produced that contained only one or the other of the alkaloids involved in the cross, while the remaining segregants contained mixtures of the two in different proportions. A secondary alkaloid that is present in small amounts in one parent may become the chief alkaloid in some individuals of a hybrid or inbred family. (2) No obvious Mendelian ratios were obtained in families segregating for different alkaloids, nor would they be expected, since the interspecific hybrids and extra chromosomal forms all show irregularities at meiosis.

ECONOMIC POSSIBILITIES

Since none of the wild species studied contained as much nicotine as the cultivated ones, *Nicotiana tabacum*, *N. rustica* and selections from crosses between these species are the most promising sources of this alkaloid. Nicotine is used widely as an insecticide; and although it is now obtained from scrap leaf material and stems of *N. tabacum* as a byproduct of the tobacco industry, new selections of

N. rustica (fig. 1, *A*) have been obtained that yield large quantities of this alkaloid and it may be possible to develop strains from them that can be grown profitably for the nicotine alone (*22*).

Anabasine is not used commercially as an insecticide in this country at present; but it has been shown experimentally to be more toxic than nicotine to *Aphis rumicis* L. (*14*). In Russia anabasine is evidently used extensively and is obtained from *Anabasis aphylla*, a shrub that grows wild in the region of the Caspian Sea (*12*). This

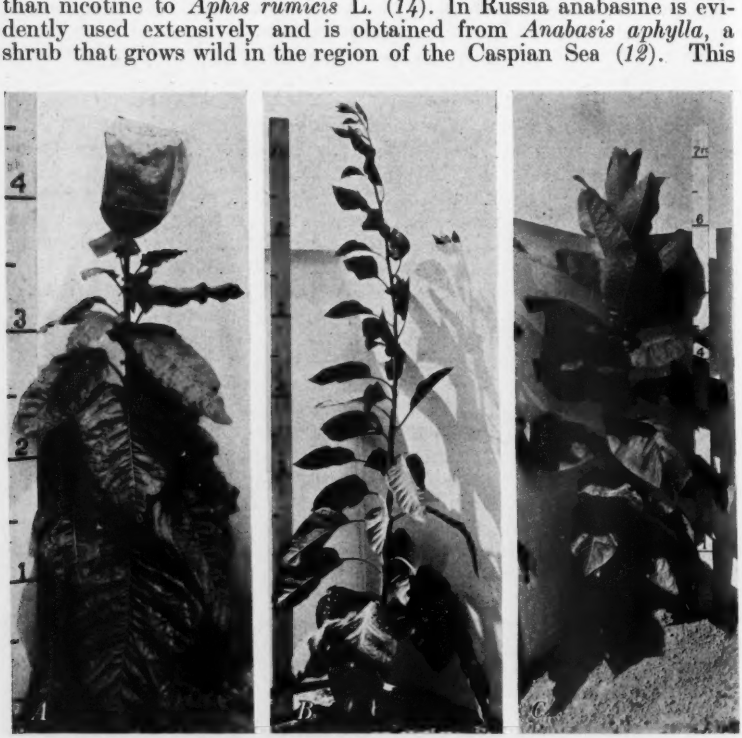

FIGURE 1.—Types of *Nicotiana* that are promising sources of the three main alkaloids found in the genus: *A*, *N. rustica*, new selection of hybrid origin, a source of nicotine; *B*, amphidiploid, *N. tabacum* × *N. glauca*, a source of anabasine; *C*, F_1, *N. tabacum* × *N. tomentosa*, a source of nornicotine.

source is not enough to supply the demand (*25*), however, and consequently there has been interest in breeding forms of *Nicotiana* having a high anabasine content.

Nicotiana glauca appears to be the best species to use for this purpose, as the plant is larger and contains a higher concentration of alkaloid than *N. debneyi*, the only other species so far determined to have anabasine as the main alkaloid. The growth habit, yield, and alkaloidal content need to be improved, however. A fairly fertile strain (fig. 1, *B*) in which some of these improvements have been made (table 2) was produced by doubling the chromosomes of the F_1 hybrid *N. tabacum* × *N. glauca* with colchicine to form an amphidiploid (*21*). Further experiments are being carried out in this

laboratory in an attempt to combine the best qualities of *N. glauca*, *N. rustica*, *N. tabacum*, and possibly *N. debneyi* into strains that will yield still larger amounts of anabasine. It is expected that fertile combinations containing full chromosome complements of any two or three or of all four species can be produced with the aid of colchicine as an agent for doubling chromosomes, provided multivalent associations at meiosis do not seriously interfere. The writers have obtained, but have not yet analyzed chemically, the following crosses and their double chromosome forms (diploid for each species): *N. debneyi* × *N. glauca*, *N. debneyi* × *N. tabacum*, *N. debneyi* × *N. glauca* × *N. rustica*, *N. debneyi* × *N. tabacum* × *N. glauca*, and *N. rustica* × *N. debneyi* × *N. tabacum*. Russian investigators have reported (*7*, *25*) that the amphidiploid and sesquidiploid ($24rr + 12g$) of crosses between *N. rustica* and *N. glauca* contained a high percentage of anabasine.

Nornicotine, the most prevalent alkaloid among wild species of *Nicotiana*, also has been shown experimentally to be highly toxic to certain insects (*14*). The F_1 hybrid *N. tabacum* × *N. tomentosa* was the largest predominantly nornicotine type that was obtained in the writers' investigations (fig. 1, *C*). Fourteen topped and suckered plants of this hybrid had an average of 1.18 percent nornicotine, an average dry leaf weight of 93.7 gm. per plant, and an estimated potential yield of 21.4 pounds of nornicotine per acre.

SUMMARY AND CONCLUSIONS

All 29 of the wild species of *Nicotiana* that were studied contained 1 or more alkaloids.

Nicotiana alata, *N. bigelovii*, *N. gossei*, and *N. wigandioides* apparently contained only nicotine.

Nicotiana glutinosa, *N. maritima*, *N. otophora*, *N. tomentosa*, and *N. trigonophylla* apparently contained only nornicotine.

Nicotiana debneyi and *N. glauca* contained largely *d,l*-anabasine. In the former about 15 percent of the total alkaloid was nicotine, whereas in the latter a much lower percentage of this alkaloid was found.

The remaining 18 species that were analyzed contained mixtures of nicotine and nornicotine. Of these, nicotine was predominant only in *N. benavidesii*, *N. langsdorffii*, and *N. stocktoni;* in *N. longiflora* and *N. plumbaginifolia* it was not possible to determine which of the two alkaloids was present in the higher concentration; and in the rest nornicotine predominated.

No wild species studied contained as much as 2 percent total alkaloidal content and most had less than 0.5 percent.

In 23 of the species, 84 to 100 percent of the total alkaloidal content was taken up by the main alkaloid.

In crosses between *Nicotiana tabacum*, which contains mostly nicotine, and species whose alkaloidal complex was made up largely or entirely of nornicotine, the hybrids contained mainly nornicotine together with small amounts of nicotine.

The F_1 and amphidiploid *Nicotiana tabacum* (nicotine) × *N. glauca* (anabasine) contained mostly anabasine with some nicotine. In one sample of this F_1 a trace of nornicotine was detected.

A quantitative method of separating anabasine from nornicotine is needed in order to study profitably the alkaloids of hybrids in which both are present.

Although the species considered as the probable wild progenitors of *Nicotiana tabacum* and *N. rustica* all have a low alkaloidal content that is predominantly nornicotine, the two cultivated species themselves have a high alkaloidal content that is mostly nicotine. A suggested explanation of this situation is that the manner of origin, selection, and culture of the cultivated species produced a high alkaloidal content, which in turn favored the formation of nicotine.

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